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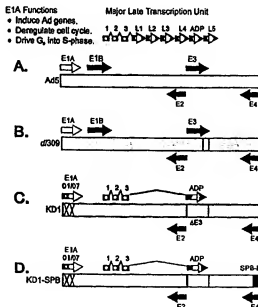
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(54) Title: REPLICATION-COMPETENT ANTI-CANCER VECTORS



(57) Abstract: Novel vectors which are replication-competent in neoplastic cells and which overexpress an adenoviral death protein are disclosed. Some of the disclosed vectors are replication-restricted to neoplastic cells or to neoplastic alveolar type II cells. Compositions and methods for promoting the death of neoplastic cells using these replication-competent vectors are also disclosed.



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### Replication-Competent Anti-Cancer Vectors

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#### 5 Background of the Invention

##### (1) Field of the Invention

This invention relates generally to the treatment of cancer and more particularly to vectors which replicate in neoplastic cells and which overexpress an adenovirus death protein (ADP) and to the use of these vectors in treating human cancer.

##### 10 (2) Description of the Related Art

Cancer is a leading cause of death in the United States and elsewhere. Depending on the type of cancer, it is typically treated with surgery, chemotherapy, and/or radiation. These treatments often fail: surgery may not remove all the cancer; some cancers are resistant to chemotherapy and radiation therapy; and chemotherapy-resistant tumors frequently develop.

##### 15 New therapies are necessary, to be used alone or in combination with classical techniques.

One potential therapy under active investigation is treating tumors with recombinant viral vectors expressing anti-cancer therapeutic proteins. Adenovirus-based vectors contain several characteristics that make them conceptually appealing for use in treating cancer, as well as for therapy of genetic disorders. Adenoviruses (hereinafter used interchangeably with

"Ads") can easily be grown in culture to high titer stocks that are stable. They have a broad host range, replicating in most human cancer cell types. Their genome can be manipulated by site-directed mutation and insertion of foreign genes expressed from foreign promoters.

- The adenovirus consists of a DNA-protein core within a protein capsid (reviewed by Stewart et al., "Adenovirus structure by x-ray crystallography and electron microscopy," in: *The Molecular Repertoire of Adenoviruses*, Doerfler, W. et al., (ed.), Springer-Verlag, Heidelberg, Germany, p. 25-38). Virions bind to a specific cellular receptor, are endocytosed, and the genome is extruded from endosomes and transported to the nucleus. The genome is a linear duplex DNA of about 36 kbp, encoding about 36 genes (Fig. 1A). In the nucleus, the "immediate early" E1A proteins are expressed initially, and these proteins induce expression of the "delayed early" proteins encoded by the E1B, E2, E3, and E4 transcription units (reviewed by Shenk, T. "Adenoviridae: the viruses and their replication" in: *Fields Virology*, Field, B.N. et al., Lippincott-Raven, Philadelphia, p. 2111-2148). E1A proteins also induce or repress cellular genes, resulting in stimulation of the cell cycle. About 23 early proteins function to usurp the cell and initiate viral DNA replication. Viral DNA replicates at about 7 h post-infection (p.i.), then late genes are expressed from the "major late" transcription unit. Major late mRNAs are synthesized from the common "major late promoter" by alternative pre-mRNA processing. Each late mRNA contains a common "tripartite leader" at its 5'-terminus (exons 1, 2, and 3 in Fig. 1), which allows for efficient translation of Ad late mRNAs. Cellular protein synthesis is shut off, and the cell becomes a factory for making viral proteins. Virions assemble in the nucleus at about 1 day p.i., and after 2-3 days the cell lyses and releases progeny virus. Cell lysis is mediated by the E3 11.6K protein, which has been renamed "adenovirus death protein" (ADP) (Tollefson et al., *J. Virol.* 70:2296-2306, 1996; Tollefson et al., *Virol.* 220:152-162, 1996). The term ADP as used herein in a generic sense refers collectively to ADP's from adenoviruses such as, e.g. Ad type 1 (Ad1), Ad type 2 (Ad2), Ad type 5 (Ad5) or Ad type 6 (Ad6) all of which express homologous ADP's with a high degree of sequence similarity.

- Human adenovirus type 5 (Ad5) is particularly useful for cancer gene therapy. It primarily causes asymptomatic or mild respiratory infections in young children, followed by long term effective immunity. Fatalities are extremely rare except when the patient is immunocompromised (Horwitz, M. S., *Adenoviruses*, p. 2149-2171 In B. N. Fields, D. M. Knipe, and P. M. Howley (eds.), *Fields Virology*, Lippincott-Raven Publishers, Philadelphia, PA, 1996). Ad5 is very well understood, can be grown in culture to high titer stocks that are stable, and can replicate in most human cancer cell types (Shenk, T., *Adenoviridae: the viruses and their replication*, p. 2111-2148. In B. N. Fields, D. M. Knipe, and P. M. Howley



(eds.), Fields Virology, Lippincott-Raven, Philadelphia, 1996). Its genome can be manipulated by site-directed mutagenesis and insertion of foreign sequences.

The Ad vectors being investigated for use in anti-cancer and gene therapy are based on recombinant Ad's that are either replication-defective or replication-competent. Typical replication-defective Ad vectors lack the E1A and E1B genes (collectively known as E1) and contain in their place an expression cassette consisting of a promoter and pre-mRNA processing signals which drive expression of a foreign gene. The E1A proteins induce transcription of other Ad genes, and in nontransformed cells they deregulate the cell cycle, induce or repress a variety of cellular genes, and force cells from G<sub>0</sub> into S-phase 48 (White, E., *Semin. Virol.* 8:505-513, 1998; Wold et al., pp. 200-232 *In* A.J. Cann (ed.), DNA Virus Replication: Frontiers in Molecular Biology, Oxford University Press, Oxford). The E1B proteins inhibit cellular apoptosis. *Id.* These vectors are unable to replicate because they lack the E1A genes required to induce Ad gene expression and DNA replication. In addition, the E3 genes are usually deleted because they are not essential for virus replication in cultured cells.

A number of investigators have constructed replication-defective Ad vectors expressing anti-cancer therapeutic proteins. Usually, these vectors have been tested by direct injection of human tumors growing in mouse models. Most commonly, these vectors express the thymidine kinase gene from herpes simplex virus, and the mice are treated with gancyclovir to kill cells transduced by the vector (see e.g., Felzmann et al., *Gene Ther.* 4:1322-1329, 1997). Another suicide gene therapy approach involves injecting tumors with a replication defective Ad vector expressing cytosine deaminase, followed by administration of 5-fluorocytosine (Topf et al., *Gene Ther.* 5:507-513, 1998). Investigators have also prepared and tested replication-defective Ad vectors expressing a cytokine-such as IL-2, IL-12, IL-6, tumor necrosis factor (TNF), type I interferons, or the co-stimulatory molecule B7-1 in the anticipation that the Ad-expressed cytokine will stimulate an immune response, including cytotoxic T-lymphocytes (CTL), against the tumor (Felzmann et al., *supra*; Putzer et al., *Proc. Natl. Acad. Sci. USA* 94:10889-10894, 1997). Other vectors express tumor antigens (e.g. melanoma MART1), proteins that de-regulate the cell cycle and induce apoptosis (p53, pRB, p21<sup>Kip1/WAF1</sup>, p16<sup>CDKN2</sup>, and even Ad E1A), and ribozymes. An Ad vector expressing FasL induces apoptosis and tumor regression of a mouse tumor (Arai et al., *Proc. Natl. Acad. Sci. USA* 94:13862-13867, 1997).

Despite these generally positive reports, it is recognized in the art that replication-defective Ad vectors have several characteristics that make them suboptimal for use in therapy. For example, production of replication-defective vectors requires that they be grown on a complementing cell line that provides the E1A proteins in trans. Such cell lines

are fastidious, and generation of virus stocks is time-consuming and expensive. In addition, although many foreign proteins have been expressed from such vectors, the level of expression is low compared to Ad late proteins.

- To address these problems, several groups have proposed using replication-competent Ad vectors for therapeutic use. Replication-competent vectors retain Ad genes essential for replication and thus do not require complementing cell lines to replicate. Replication-competent Ad vectors lyse cells as a natural part of the life cycle of the vector. Another advantage of replication-competent Ad vectors occurs when the vector is engineered to encode and express a foreign protein. Such vectors would be expected to greatly amplify synthesis of the encoded protein *in vivo* as the vector replicates. However, in order to prevent RC vectors from damaging normal tissues and causing disseminated viremia, it is important that they have some feature that limits their replication to cancer cells.

- Wyeth Laboratories developed replication-competent Ad vectors for vaccination purposes, using vaccine strains of Ad serotypes 4, 7, and 5 (Lubeck et al., *AIDS Res. Hum. Retroviruses* 10:1443-1449, 1994). Foreign genes were inserted into the E3 region (with the E3 genes deleted) or into a site at the right end of the genome. Two foreign genes used were hepatitis B surface antigen and the HIV envelope protein. They obtained good expression in culture, and were able to raise antisera in animal models. Phase I human trials were ambiguous, and the project was mostly abandoned.

- Onyx Pharmaceuticals recently reported on adenovirus-based anti-cancer vectors which are replication deficient in non-neoplastic cells but which exhibit a replication phenotype in neoplastic cells lacking functional p53 and/or retinoblastoma (pRB) tumor suppressor proteins (U.S. Patent No. 5,677,178; Heise et al., *Nature Med.* 6:639-645, 1997; Bischoff et al., *Science* 274:373-376, 1996). This phenotype is reportedly accomplished by using recombinant adenoviruses containing a mutation in the E1B region that make the encoded E1B-55K protein incapable of binding to p53 and/or a mutation(s) in the E1A region which make the encoded E1A protein (p289R or p243R) incapable of binding to pRB and/or the cellular 300 kD polypeptide and/or the 107 kD polypeptide. E1B-55K has at least two independent functions: it binds and inactivates the tumor suppressor protein p53, and it is required for efficient transport of Ad mRNA from the nucleus. Because these E1B and E1A viral proteins are involved in forcing cells into S-phase, which is required for replication of adenovirus DNA, and because the p53 and pRB proteins block cell cycle progression, the recombinant adenovirus vectors described by Onyx should replicate in cells defective in p53 and/or pRB, which is the case for many cancer cells, but not in cells with wild-type p53 and/or pRB. Onyx has reported that replication of an adenovirus lacking E1B-55K, which is named ONYX-015, was restricted to p53-minus cancer cell lines (Bischoff et al., *supra*), and

that ONYX-015 slowed the growth or caused regression of a p53-minus human tumor growing in nude mice (Heise et al., *supra*). Others have challenged the Onyx report claiming that replication of ONYX-015 is independent of p53 genotype and occurs efficiently in some primary cultured human cells (Harada and Berk, *J. Virol.* 73:5333-5344, 1999). It is now  
5 known that ONYX-015 can replicate in cells with wild-type p53 (Goodrum et al., *J. Virol.* 72:9479-9490, 1998; Harada et al., *J. Virol.* 73:5333-5344, 1999; Hay et al., *Hum. Gene Ther.* 10:579-590, 1999; Rothmann et al., *J. Virol.* 72:9470-9478, 1998; Turnell et al., *J. Virol.* 73:2074-2083, 1999). ONYX-015 does not replicate as well as wild-type adenovirus because E1B-55K is not available to facilitate viral mRNA transport from the nucleus. Also, ONYX-  
10 015 expresses less ADP than wild-type virus (see Example 1 below).

As an extension of the ONYX-015 concept, a replication-competent adenovirus vector was designed that has the gene for E1B-55K replaced with the herpes simplex virus thymidine kinase gene (Wilder et al., *Gene Therapy* 6:57-62, 1999). The group that  
15 constructed this vector reported that the combination of the vector plus gancyclovir showed a therapeutic effect on a human colon cancer in a nude mouse model (Wilder et al., *Cancer Res.* 59:410-413, 1999). However, this vector lacks the gene for ADP, and accordingly, the vector will lyse cells and spread from cell-to-cell less efficiently than an equivalent vector that expresses ADP. The gene for ADP is also lacking in another replication-competent adenovirus vector that has been described, in which a minimal enhancer/promoter of the  
20 human prostate specific antigen was inserted into the adenovirus E1A enhancer/promoter (Rodriguez et al., *Cancer Res.* 57:2559-2563, 1997).

Another strategy for replication-competent vector improvement is to place replication under the control of tissue-specific promoters. One group replaced the basal E1A promoter with a modified promoter for  $\alpha$ -fetoprotein (AFP) (Hallenbeck et al., *Hum. Gene Ther.*  
25 10:1721-1733, 1999). AFP is expressed in the liver during development, but it is not expressed in adults. However, it is expressed in 70-80% of patients with hepatocellular carcinoma. Growth of this vector was limited to AFP-expressing cells and the vector showed some suppression of xenotransplants. *Id.* A series of RC vectors has also been developed that have expression of the E1A and E1B genes dependent on the prostate tumor-specific  
30 prostate specific antigen (PSA) and kallikrein promoters/enhancers (Rodriguez et al., *Cancer Res.* 60:1196, 1997; Yu et al., *Cancer Res.* 59:4200-4203, 2000; Yu et al., *Cancer Res.* 59:1498-1504, 1999).

Thus, there is a continuing need for vectors that replicate and spread efficiently in tumors but that can be modified such that they replicate poorly or not at all in normal tissue.

35 Summary of the Invention

Briefly, therefore, the present invention is directed to novel vectors which are replication competent in neoplastic cells and which overexpress an adenovirus death protein (ADP). The work reported herein demonstrates the discovery that overexpression of ADP by a recombinant adenovirus allows the construction of a replication-competent adenovirus that kills neoplastic cells and spreads from cell-to-cell at a rate similar to or faster than that exhibited by adenoviruses expressing wild-type levels of ADP, even when the recombinant adenovirus contains a mutation that would otherwise reduce its replication rate in non-neoplastic cells. This discovery was unexpected because it could not have been predicted from what was known about adenovirus biology that Ad vectors overexpressing ADP remain viable and that the infected cells are not killed by the higher amounts of ADP before the Ad vector produces new virus particles that can spread to other tumor cells. Indeed, naturally-occurring adenoviruses express ADP in low amounts from the E3 promoter at early stages of infection, and begin to make ADP in large amounts only at 24-30 h p.i., once virions have been assembled in the cell nucleus. It is believed that other non-adenoviral vectors can be used to deliver ADP's cell-killing activity to neoplastic cells, including other viral vectors and plasmid expression vectors.

Thus, in one preferred embodiment, the ADP-expressing vector comprises a recombinant adenovirus lacking expression of at least one E3 protein selected from the group consisting of: gp19K; RID $\alpha$  (also known as 10.4K); RID $\beta$  (also known as 14.5K) and 14.7K. Because these E3 proteins inhibit immune-mediated inflammation and/or apoptosis of Ad-infected cells, it is believed that a recombinant adenovirus lacking one or more of these E3 proteins will stimulate infiltration of inflammatory and immune cells into a tumor treated with the adenovirus and that this host immune response will aid in destruction of the tumor as well as tumors that have metastasized. The ADP expressed by preferred embodiments comprises a naturally-occurring amino acid sequence from a human adenovirus of subgroup C, namely Ad1, Ad2, Ad5 and Ad6.

In another embodiment, replication of the vector is restricted to neoplastic cells. Such replication-restricted vectors are useful in treating cancer patients in which it is desirable to eliminate or reduce damage to normal cells and tissues that might be caused by the vector, particularly viral vectors that kill the host cell as part of their life cycle. In preferred embodiments, a recombinant adenovirus has a replication-restricted phenotype because the recombinant adenovirus is incapable of expressing an E1A viral protein which binds the pRB and the p300/CBP proteins or because the E4 promoter has been substituted with a promoter that is activated only in neoplastic cells and/or cells of a specific tissue.

In yet another embodiment, the invention provides a vector which overexpresses ADP and whose replication is under the control of a tissue specific promoter, tumor specific

promoter or an inducible promoter. In preferred embodiments, the vector comprises a recombinant adenovirus in which the tissue specific promoter or inducible promoter is substituted for the E4 promoter. Such vectors are useful for restricting replication of the vector and its ADP-mediated cell killing to cells of a particular type or to cells exposed to an exogenous agent that activates the promoter. A preferred tissue-specific or inducible vector also expresses a phenotype that restricts its replication to neoplastic cells.

In yet another embodiment, the invention provides a vector which overexpresses ADP but which is not restricted to tumors by a specific genetic modification. Such a vector is more destructive to neoplastic cells than even the naturally occurring Ad's of subgroup C. In preferred embodiments, this vector could be used for patients with terminal cancer not treatable by another method, and who have pre-existing neutralizing antibodies to Ad or to which neutralizing antibodies can be administered.

In still another embodiment, the invention provides a composition comprising a first recombinant virus which is replication competent in a neoplastic cell and overexpresses the adenovirus death protein. In one embodiment, the recombinant virus is contained within a delivery vehicle comprising a targeting moiety that limits delivery of the virus to cells of a certain type. With this embodiment, the replication-competent vector can be of any ADP-overexpressing configuration described herein. In some embodiments, the composition also comprises a second recombinant virus which is replication-defective and which expresses an anti-cancer gene product. In some embodiments, the replication-defective vector may be engineered to overexpress ADP when replication of this vector is complemented by a replication-competent vector. The recombinant virus complements spread of the replication-defective virus, as well as its encoded anti-cancer product, throughout a tumor. In preferred embodiments, the first recombinant virus is a recombinant adenovirus whose replication is restricted to neoplastic cells and/or which lacks expression of one or more of the E3 gp19K; RID $\alpha$ ; RID $\beta$ ; and 14.7K proteins.

In additional embodiments, the invention provides replication-competent vectors that overexpresses an ADP and also expresses an anti-cancer product. As with previous embodiments, the vector can be of any ADP-overexpressing configuration provided herein. Preferably, replication of the virus is engineered to (a) be restricted to neoplastic cells, e.g., by replacing the E4 promoter with a tissue specific or tumor specific promoter and/or (b) lack expression of one or more of the E3 gp19K; RID $\alpha$ ; RID $\beta$ ; and 14.7K proteins. In some embodiments, the anti-cancer product is inserted into the E3 region.

The ADP-expressing vectors and compositions of the invention are useful in a method for promoting death of a neoplastic cell. The method comprises contacting the neoplastic cell with a vector which is replication-competent in the neoplastic cell and which

overexpresses ADP. Where the neoplastic cell comprises a tumor in a patient, the vector is administered directly to the tumor or, in other embodiments, the vector is administered to the patient systemically or in a delivery vehicle containing a targeting moiety that directs delivery of the vector to the tumor. In embodiments where the vector is a recombinant virus, the method can also comprise passively immunizing the patient against the virus.

In yet another embodiment of the invention, the vector may be used in combination with radiation therapy. The radiation therapy can be any form of radiation therapy used in the art such as for example, external beam radiation such as x-ray treatment, radiation delivered by insertion of radioactive materials within the body near or at the tumor site such as treatment with gamma ray emitting radionuclides, particle beam therapy which utilizes neutrons or charged particles and the like. In addition, this embodiment encompasses the use of more than one of the vectors of the present invention in a cocktail in combination with radiation therapy.

Another embodiment of the invention involves the use of the recombinant vector in combination with chemotherapy as has been disclosed for other adenovirus vectors (U.S. Patent No. 5,846,945). Chemotherapeutic agents are known in the art and include antimetabolites including pyrimidine-analogue and purine-analogue antimetabolites, plant alkaloids, antitumor antibiotics, alkylating agents and the like. The use of more than one of the vectors of the present invention with a chemotherapeutic agent or agents is also contemplated within this embodiment.

Among the several advantages found to be achieved by the present invention, therefore, may be noted the provision of replication-competent vectors, particularly viruses, which rapidly kill cancer cells and spread from cell-to-cell in a tumor; the provision of such vectors whose replication can be induced or which is restricted to tumors and/or to cells of a certain tissue type; and the provision of compositions and methods for anti-cancer therapy which cause little to no side effects in normal tissues.

#### Brief Description of the Drawings

Figure 1 is a schematic of gene expression in Ad5 (Fig. 1A) and KD3, a preferred embodiment of the invention (Fig. 1B), in which the respective genomes are represented by the stippled bars and transcription units represented by arrows above and below the bars, with the E3 proteins listed above the arrows for the E3 transcription unit, and the L1 to L5 families of late mRNA's indicated.

Figure 2 illustrates the overexpression of ADP by KD1, KD3, GZ1, and GZ3 showing an immunoblot of proteins isolated from human A549 cells infected with the indicated viruses and probed with an anti-ADP antibody, with ADP indicating differently glycosylated and proteolytically processed forms of ADP.

Figure 3 illustrates that the E1A *d1101/1107* mutation referred to in the figure and hereinafter as *d101/07*, retards expression of late proteins, showing an immunoblot of E1A proteins and late proteins in A549 cells infected with the indicated viruses in the absence (Figs. 3A and 3B) or presence (Figs. 3C and 3D) of *d327*, which has a wild-type E1A region and has a deletion of all E3 genes but the gene encoding the 12.5K protein (Figs. 3C and 3D). An antiserum specific to the E1A proteins was used for Fig. 3A and 3C. An antiserum raised against Ad5 virions was used for Figs. 3B and 3D.

Figure 4 illustrates that KD1 and KD3 kill cells more efficiently than control viruses that express less or no ADP, showing a graph of the percent of A549 cells infected with the indicated viruses that were viable at the indicated days p.i. as determined by trypan blue exclusion.

Figure 5 is a cell spread assay illustrating that overexpression of ADP enhances spread of virus from cell to cell, showing monolayers infected with the indicated viruses at the indicated PFU/cell which were treated at 7 days p.i. with crystal violet, which stains live cells but not dead cells.

Figure 6 illustrates that KD1 and KD3 replicate well in growing cells but not in growth-arrested cells showing the virus titer extracted from growing or growth arrested HEL-229 cells at various times following infection with 100 PFU/ml of the following viruses: *d309* (Fig. 6A), *d101/07* (fig. 6B), KD1 (Fig. 6C) and KD3 (Fig 6D).

Figure 7 illustrates that KD1 and KD3 are defective in killing primary human bronchial epithelial cells showing these cell monolayers infected at 30% confluency with 10 PFU/ml of the indicated viruses and stained at 5 days p.i. with neutral red.

Figure 8 illustrates that KD1 and KD3 reduce the growth rate of human A549 cell tumors growing in nude mice, showing in Fig. 8A a graph of average-fold increase in tumor size plotted against the number of weeks following infection of the tumor with buffer or with  $5 \times 10^7$  PFU at weekly intervals of or the indicated viruses, and showing in Fig. 8B a similar graph of tumors injected once with  $5 \times 10^8$  PFU of KD3 or GZ3.

Figure 9 illustrates that KD1 and KD3 reduce the growth rate of human Hep3B cell tumors growing in nude mice, showing a graph of average-fold increase in tumor size plotted against the number of weeks following injection of the tumor with buffer or with  $5 \times 10^7$  PFU of *d309*, KD1 or KD3 at twice weekly intervals of the indicated viruses.

Figure 10 illustrates that KD1 and KD3 complement the replication and spread of Ad- $\beta$ -gal, a replication-defective vector that expresses  $\beta$ -galactosidase, using an infectious center assay showing in Fig. 10A a picture of A549 cell monolayers seeded with A549 cells infected with Ad- $\beta$ -gal alone or with the indicated viruses, with Figs 10B and 10C showing close-up views of two of the monolayers of Fig. 10A.

Figure 11 is a bar graph illustrating that KD1 and KD3 increase the expression of luciferase in human Hep3B cell tumors growing in nude mice, using an assay in which tumors were injected with the indicated combinations of viruses, then were extracted 2 weeks p.i. and assayed for luciferase activity. The numbers in parentheses indicated the fold increase in luciferase activity compared to that of the Adluc vector plus buffer.

Figure 12 is a graph showing the results of a standard plaque development assay for KD1 and KD1-SPB on A549 cells engineered to express the TTF1 transcription factor (A549/TTF1) and the parental 549 cells, in which data are plotted as the number of plaques observed on a particular day in the assay divided by the final number of plaques observed for that virus multiplied by 100.

Figure 13 is a cell spread assay for KD1 and KD1-SPB on H441 cells and Hep3B cells, where cells were infected with the indicated amounts of KD1 or KD1-SPB and H441 cells and Hep3B cells were stained with crystal violet at 5 days p.i. and 8 days p.i., respectively.

Figure 14 is a graph showing the results of a standard plaque development assay for *dl309* and two preferred embodiments of the invention, GZ1 and GZ3, in which data are plotted as the number of plaques observed on a particular day in the assay divided by the final number of plaques observed for that virus multiplied by 100.

Figure 15 is a cell spread assay illustrating that the combination of KD1, KD3, GZ1, or GZ3 with x-ray radiation is more effective in destroying A549 cell monolayers than is virus vector alone or radiation alone, wherein cells were infected with the indicated amounts of the indicated viruses, radiated with 600 centigrays (cGy) of x-radiation (bottom panel), or mock radiated (top panel), then stained with crystal violet at 6 days p.i.

Figure 16 is a graph of a cell spread assay illustrating that  $10^{-3}$  PFU of KD1, KD3, GZ1, or GZ3 used in combination with 150, 300, or 600 centigrays of radiation is more effective in destroying A549 cell monolayers than virus vector alone or radiation alone. Cell viability is based on the amount of crystal violet extracted from the culture wells, using the mock-infected non-radiated well as 100% viability.

Figure 17 illustrates that the combination of KD3 or GZ3 plus x-ray radiation is more effective in reducing the growth of A549 cell tumors growing in nude mice than KD3 alone or GZ3 alone.

Figure 18 illustrates a structure-function analysis of ADP, showing in Fig. 18A the amino acid sequence of the adenovirus death protein encoded by Ad2, with the various putative domains and glycosylation sites labeled and showing in Fig. 18B a schematic of the ADP gene in *rec700* and in the indicated deletion mutants, with the right column



summarizing the death promoting phenotype of the various mutants as a percentage of the wild-type phenotype.

Figures 19A and 19B illustrate a cell viability assay of the indicated ADP mutants showing a graph of viability as determined by trypan blue exclusion plotted against hours (Fig. 19A) or days (Fig. 19B) postinfection.

Figure 20 depicts the amino acid sequence, shown in single letter code, for the ADP proteins of Ad1, Ad2, Ad5, and Ad6 (SEQ ID NOS:5-8), for the Ad2 ADP mutants *d1716*, *d1715*, *d1714*, and *d1737* (SEQ ID NOS:9-12), and for the putative luminal domain (SEQ ID NO:17), the transmembrane domain (SEQ ID NO:18), the cytosolic basic-proline domain (SEQ ID NO:19), and the remainder of the cytosolic domain (SEQ ID NO:20) of the ADP protein of Ad2.

Figure 21 presents the complete nucleotide sequence of the genome of Ad5.

Figure 22 presents the complete nucleotide sequence of the genome of KD1 (SEQ ID NO:1).

Figure 23 presents the complete nucleotide sequence of the genome of KD3 (SEQ ID NO:2).

Figure 24 is a schematic of the following vectors: A. Ad5. The stippled bar indicates the DNA genome of 36 kbp. The open arrow indicates the immediate early E1A transcription unit, and the black arrows are the delayed early E1B, E2, E3, and E4 transcription units. The hatched arrows indicate the five families of major late mRNAs, and also the ADP mRNA, which is synthesized as part of the major late transcription unit. Each major late mRNA has a tripartite leader (leaders 1, 2, and 3) spliced to its 5' terminus. B. dl309. dl309 is identical to Ad5 except it has the E3-RID and E3-14.7K genes deleted. dl309 expresses ADP at levels similar to Ad5. C. KD1. KD1 has two small deletions (indicated by "X" marks) in the E1A gene that abolish binding of the E1A proteins to pRB or p300/CBP. It lacks all E3 genes except adp. ADP is expressed earlier in infection and in greater abundance than is ADP from Ad5 or dl309 Doronin et al., *J. Virol.* 74:6147-6155. D. KD1-SPB. KD1-SPB is identical to KD1, except it has the E4 promoter replaced by the promoter for Surfactant Protein B (SPB-P).

Figure 25 presents graphs illustrating that KD1-SPB grows as well as KD1 in H441 lung carcinoma cells but much more poorly than KD1 in Hep 3B hepatoma cells. CsCl-banded stocks of KD1-SPB and KD1 were titrated using standard methods (Tollefson et al., p. 1-9 In W.S.M. Wold (ed.), *Adenovirus Methods and Protocols*. Humana Press, Inc., Totowa, NJ, 1998) on 293-E4 or 293 cells (A), or on A549 cells (B). The data are plotted as the number of plaques seen on any day of the plaque assay as a percentage of the number of plaques seen on the final day of the assay (Tollefson et al., *Virology* 220:152-162, 1996).

Figure 26 presents micrographs illustrating that KD1-SPB induces CPE in H441 cells but not Hep 3B cells. H441 and Hep 3B monolayers were mock-infected or infected with 10 PFU/cell of KD1 or KD1-SPB, then photographed under phase contrast at 4 or 7 days p.i.

Figure 27 depicts Southern hybridizations and a graph illustrating that KD1-SPB DNA is synthesized efficiently in H441 but not Hep 3B cells. H441 or Hep 3B cells were infected with 10 PFU/cell of KD1 or KD1-SPB. Total genomic DNA was isolated at 0, 5, 24, 48, 72, and 96 h p.i., digested with HindIII, resolved by agarose gel electrophoresis, blotted, and hybridized with <sup>32</sup>P-labeled Ad DNA. A. Autoradiogram. B. PhosphorImager quantitation of the DNA bands in Panel A.

Figure 28 presents graphs depicting single step growth curves showing that KD1-SPB grows well in H441 but not Hep 3B cells. Cells were infected with 10 PFU/cell of KD1 or KD1-SPB. Vectors were extracted at the indicated days p.i. and titers determined by plaque assay.

Figure 29 depicts immunoblots showing that KD1-SPB expresses E4ORF3 and ADP in H441 but not Hep 3B cells. Cells were infected with 10 PFU/cell of KD1 or KD1-SPB. At 24 h p.i., protein extracts were analyzed for E1A, E4ORF3, and ADP using specific antisera. The E1A proteins appear as multiple bands. ADP appears as two bands; the upper band is glycosylated and the lower band is a proteolytically cleaved species (Scaria et al., *Virology* 191:743-753, 1992; Tollefson et al., *J. Virol.* 66:3633-3642).

Figure 30 depicts immunofluorescence micrographs showing that KD1-SPB expresses E4ORF3 in H441 but not Hep 3B cells. Cells growing on coverslips were infected with 20 PFU/cell of KD1, KD1-SPB, or d1309 (wild-type). At 48 h (Panel A) or 6 days (Panel B), cells were fixed and stained with a rabbit polyclonal antipeptide antiserum against E4ORF3. Photographs were taken using a 100X Planapo lens. Each panel shows about 8 nuclei. This figure is part of the same experiment shown in Figure 31.

Figure 31 depicts immunofluorescence micrographs showing that KD1-SPB does not express E2-DBP or fiber efficiently in Hep 3B cells. Hep 3B cells were infected with 20 PFU/cell of KD1-SPB or KD1. At 48 h (A) or 6 days (B) p.i., cells were fixed and double-stained using a rabbit polyclonal antiserum against DBP and a mouse monoclonal antibody against fiber. The same fields are shown for DBP and fiber. This figure is part of the same experiment shown in Figure 30.

Figure 32 presents graphs illustrating that KD1-SPB lyses H441 but not Hep 3B as efficiently as KD1. H441 or Hep 3B cells were mock-infected or infected with 20 PFU/cell of KD1 or KD1-SPB. Cell lysis was determined by release of lactate dehydrogenase from the cells into the medium.

Figure 33 presents graphs illustrating that KD1-SPB suppresses growth of H441 tumors in nude mice equally as well as KD1. Tumor cells were injected into flanks of nude mice and allowed to grow to about 100  $\mu$ l (H441) or 150  $\mu$ l (Hep 3B) volumes. Tumors (n = 10) were injected with DMEM (mock) or with  $5 \times 10^7$  PFU of KD1 or KD1-SPB. Injections of the viruses were repeated twice weekly for 3 weeks to a total dose of  $3.0 \times 10^8$  PFU per tumor. Tumors were measured and the mean fold-increase in tumor size was calculated.

#### Description of the Preferred Embodiments

In accordance with the present invention, it has been discovered that overexpression of ADP by a recombinant adenovirus results in faster lysis of cells and spread of the virus throughout a cell monolayer than viruses expressing wild-type levels of ADP. It has also been discovered that this function for ADP is manifest in an adenovirus that contains E1A mutations that restrict adenoviral replication to neoplastic cells. Thus, vectors which are both replication competent in neoplastic cells and which overexpress ADP should be useful in anti-cancer therapy.

In the context of this disclosure, the following terms will be defined as follows unless otherwise indicated:

"Naturally-occurring" as applied to an object such as a polynucleotide, polypeptide, or virus means that the object can be isolated from a source in nature and has not been intentionally modified by a human.

"Neoplastic cell" means a cell which exhibits an aberrant growth phenotype characterized by a significant loss of control of cell proliferation and includes actively replicating cells as well as cells in a temporary non-replicative resting state ( $G_1$  or  $G_2$ ). A neoplastic cell may have a well-differentiated phenotype or a poorly-differentiated phenotype and may comprise a benign neoplasm or a malignant neoplasm.

"Recombinant virus" means any viral genome or virion that is different than a wild-type virus due to a deletion, insertion, or substitution of one or more nucleotides in the wild-type viral genome. The recombinant virus can have changes in the number of amino acid sequences encoded and expressed or in the amount or activity of proteins expressed by the virus. In particular, the term includes recombinant viruses generated by the intervention of a human.

"Replication-competent" as applied to a vector means that the vector is capable of replicating in normal and/or neoplastic cells. As applied to a recombinant virus, "replication-competent" means that the virus exhibits the following phenotypic characteristics in normal and/or neoplastic cells: cell infection; replication of the viral genome; and production and release of new virus particles; although one or more of these characteristics need not occur at the same rate as they occur in the same cell type infected by a wild-type virus, and may occur

at a faster or slower rate. Where the recombinant virus is derived from a virus such as adenovirus that lyses the cell as part of its life cycle, it is preferred that at least 5 to 25% of the cells in a cell culture monolayer are dead 5 days after infection. Preferably, a replication-competent virus infects and lyses at least 25 to 50%, more preferably at least 75%, and most preferably at least 90% of the cells of the monolayer by 5 days post infection (p.i.).

"Replication-defective" as applied to a recombinant virus means the virus is incapable of, or is greatly compromised in, replicating its genome in any cell type in the absence of a complementing replication-competent virus. Exceptions to this are cell lines such as 293 cells that have been engineered to express adenovirus E1A and E1B proteins.

"Replication-restricted" as applied to a vector of the invention means the vector replicates better in a dividing cell, i.e. either a neoplastic cell or a non-neoplastic, dividing cell, than in a cell of the same type that is not neoplastic and/or not dividing, which is also referenced herein as a normal, non-dividing cell. Preferably, a replication-restricted virus kills at least 10% more neoplastic cells than normal, non-dividing cells in cell culture monolayers of the same size, as measured by the number of cells showing cytopathic effects (CPE) at 5 days p.i. More preferably, between 25% and 50%, and even more preferably, between 50% and 75% more neoplastic than normal cells are killed by a replication-restricted virus. Most preferably, a replication-restricted adenovirus kills between 75% and 100% more neoplastic than normal cells in equal sized monolayers by 5 days p.i.

In one embodiment the invention provides a vector that is replication-competent in neoplastic cells and which overexpresses an ADP. Vectors useful in the invention include but are not limited to plasmid-expression vectors, bacterial vectors such as *Salmonella* species that are able to invade and survive in a number of different cell types, vectors derived from DNA viruses such as human and non-human adenoviruses, adenovirus associated viruses (AAVs), poxviruses, herpesviruses, and vectors derived from RNA viruses such as retroviruses and alphaviruses. Preferred vectors include recombinant viruses engineered to overexpress an ADP. Recombinant adenoviruses are particularly preferred for use as the vector, especially vectors derived from Ad1, Ad2, Ad5 or Ad6.

Vectors according to the invention overexpress ADP. As applied to recombinant Ad and AAV vectors, the term "overexpresses ADP" means that more ADP molecules are made per viral genome present in a dividing cell infected by the vector than expressed by any previously known recombinant adenoviral vector or AAV in a dividing cell of the same type. As applied to other, non-adenoviral vectors, "overexpresses ADP" means that the virus expresses sufficient ADP to lyse a cell containing the vector.

Vectors overexpressing ADP can be prepared using routine methodology. See, e.g., *A Laboratory Cloning Manual*, 2nd Ed., vol. 3, Sambrook et al., eds., Cold Spring Harbor

Laboratory Press, 1989. For example, a polynucleotide encoding the ADP can be cloned into a plasmid expression vector known to efficiently express heterologous proteins in mammalian cells. The polynucleotide should also include appropriate termination and polyadenylation signals. Enhancer elements may also be added to the plasmid to increase the amount of ADP expression. Viral vectors overexpressing ADP can be prepared using similar materials and techniques.

Where the virus is a recombinant adenovirus, overexpression of ADP can be achieved in a multitude of ways. In general, any type of deletion in the E3 region that removes a splice site for any of the E3 mRNAs will lead to overexpression of the mRNA for ADP, inasmuch as more of the E3 pre-mRNA molecules will be processed into the mRNA for ADP. This is exemplified in the KD1, KD3, GZ1 and GZ3 vectors (SEQ ID NOS:1-4) whose construction is described below. Other means of achieving overexpression of ADP in Ad vectors include, but are not limited to: insertion of pre-mRNA splicing and cleavage/polyadenylation signals at sites flanking the gene for ADP; expression of ADP from another promoter, e.g. the human cytomegalovirus promoter, inserted into a variety of sites in the Ad genome; and insertion of the gene for ADP behind the gene for another Ad mRNA, together with a sequence on the 5' side of the ADP sequence that allows for internal initiation of translation of ADP, e.g. the Ad tripartite leader or a viral internal ribosome initiation sequence.

The ADP expressed by a vector according to the invention is any polypeptide comprising a naturally-occurring full-length ADP amino acid sequence or variant thereof that confers upon a vector expressing the ADP the ability to lyse a cell containing the vector such that replicated copies of the vector are released from the infected cell. A preferred full-length ADP comprises the ADP amino acid sequence encoded by Ad1, Ad2, Ad5 or Ad6. These naturally-occurring ADP sequences are set forth in SEQ ID NOS:5-8, respectively. ADP variants include fragments and deletion mutants of naturally-occurring adenovirus death proteins, as well as full-length molecules, fragments and deletion mutants containing conservative amino acid substitutions, provided that such variants retain the ability, when expressed by a vector inside a cell, to lyse the cell.

Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. Conservatively substituted amino acids can be grouped according to the chemical properties of their side chains. For example, one grouping of amino acids includes those amino acids having neutral and hydrophobic side chains (A, V, L, I, P, W, F, and M); another grouping is those amino acids having neutral and polar side chains (G, S, T, Y, C, N, and Q); another grouping is those amino acids having basic side chains (K, R, and H); another grouping is those amino acids having acidic side chains (D and E); another grouping is those amino acids having aliphatic side chains (G, A, V, L, and I); another

grouping is those amino acids having aliphatic-hydroxyl side chains (S and T); another grouping is those amino acids having amine-containing side chains (N, Q, K, R, and H); another grouping is those amino acids having aromatic side chains (F, Y, and W); and another grouping is those amino acids having sulfur-containing side chains (C and M). Preferred conservative amino acid substitutions groups are: R-K; E-D, Y-F, L-M; V-I, and Q-H.

As used herein, an ADP variant can also include modifications of a naturally-occurring ADP in which one or more amino acids have been inserted, deleted or replaced with a different amino acid or a modified or unusual amino acid, as well as modifications such as glycosylation or phosphorylation of one or more amino acids so long as the ADP variant containing the modified sequence retains cell lysing activity.

As described below, the inventors herein performed a structure-function analysis of ADP that defined specific domains in ADP required to promote cell death. Using this information, when combined with known recombinant DNA and cloning methodology, it is believed the skilled artisan can readily construct ADP variants of a naturally-occurring adenovirus death protein and test them for cell lysing activity. A preferred ADP deletion mutant comprises an ADP amino acid sequence from any of the deletion mutants *dI716*, *dI715*, *dI714* and *dI737*, whose ADP sequences are set forth in SEQ ID NOS:9-12, respectively).

Where the vector is derived from a virus, it is preferred that the virus lack expression of one or more viral proteins involved in avoiding host anti-viral defenses such as immune-mediated inflammation and/or apoptosis of infected cells. For example, adenovirus contains a cassette of genes that prevents killing of Ad-infected cells by the immune system (Wold et al., *Semin. Virol.*, 1998 (8:515-523, 1998). The E3-14.7K protein and the E3 RID (Receptor Internalization and Degradation) protein, which is a complex consisting of RID $\alpha$  and RID $\beta$ , inhibit apoptosis of Ad-infected cells induced by tumor necrosis factor (TNF) and the Fas ligand which are expressed on, or secreted by, activated macrophages, natural killer (NK) cells, and cytotoxic lymphocytes (CTLs) (Tollefson et al., *Nature* 392:727-730, 1998). The E3-gp19K protein inhibits CTL-killing of infected cells by blocking transport of MHC class I antigens to the cell surface (Wold et al., *supra*). Thus, it is believed that infection of tumor cells by such viral vectors will stimulate infiltration of inflammatory cells and lymphocytes into the tumor, and will not prevent infected tumor cells from apoptosis induced by cytolytic cells of the immune system, or against apoptosis inducing cytokines. For example, it is known that when mice are infected with Ad mutants lacking the E3 gp19K, RID and 14.7K proteins there is a dramatic increase (as compared to E3-positive Ad) in infiltration of inflammatory cells and lymphocytes into the infected tissue (Sparer et al., *J. Virol.* 70:2431-2439, 1996). A similar infiltration of tumors infected by an ADP-expressing viral vector of

the invention would be expected to further promote destruction of the tumor by adding an immune system attack to the ADP-mediated killing activity. For example, it is believed that the viral infection will stimulate formation of tumor-specific CTL's that can kill neoplastic cells not only in the tumor but also ones that have metastasized. In addition, it is also  
5 expected that vector-specific CTL's will be generated which could attack vector-infected cells if the vector spreads away from the tumor into normal cells. Because viral vectors overexpressing ADP will spread rapidly through the tumor, it is believed these immune mechanisms will have little effect on spread of the vector.

Where the vector is a recombinant adenovirus, it is preferred that the adenovirus lack  
10 expression of each of the E3 gp19K, RID, and 14.7K proteins. By "lack expression" and "lacking expression" of a protein(s), it is meant that the viral genome contains one or more mutations that inactivates expression of a functional protein, i.e., one having all the functions of the wild-type protein. The inactivating mutation includes but is not limited to substitution or deletion of one or more nucleotides in the encoding gene(s) that prevents expression of  
15 functional transcripts or that results in transcripts encoding nonfunctional translation products. A particularly preferred way to inactivate expression of the Ad E3 gp19K, RID, and 14.7K proteins is by deleting the E3 region containing the genes encoding these proteins. Preferably, one or both of the E3 genes encoding the E3 6.7K and 12.5K proteins are also deleted because, as discussed in the Examples below, it is believed that deletion of most or all  
20 of the E3 genes other than the ADP gene facilitates overexpression of ADP mRNA by reducing competition for splicing of the major late pre-mRNAs. Preferred Ad vectors containing an E3 deletion that overexpress ADP are GZ1 (SEQ ID NO:3) and GZ3 (SEQ ID NO:4), whose construction and properties are described in the Examples below.

The invention also provides ADP-expressing vectors whose replication is restricted to  
25 dividing cells. Any means known to provide such a replication-restricted phenotype may be used. For example, WO 96/40238 describes microbes that preferentially invade tumor cells as well as methods for identifying and isolating bacterial promoters that are selectively activated in tumors. It is also contemplated that expression of one or more vector proteins essential for replication can be placed under the control of the promoter for a cellular gene  
30 whose expression is known to be upregulated in neoplastic cells. Examples of such genes include but are not limited to: the breast cancer markers mammaglobin (Watson et al., *Oncogene* 16:817-824, 1998); BRCA1 (Norris et al., *J. Biol. Chem.* 270:22777-22782, 1995) *her2/neu* (Scott et al., *J. Biol. Chem.* 269:19848-19858, 1994); prostate specific antigen (U.S. Patent 5,698,443); surfactant protein B for lung alveoli (Yan et al., *J. Biol. Chem.* 270:24852-  
35 24857, 1995); factor VII for liver (Greenberg et al., *Proc. Natl. Acad. Sci. USA* 92:12347-12351, 1995); and survivin for cancer in general (Li et al., *Nature* 396:580-584). Where the

vector is an adenovirus, it is contemplated that such tumor-specific promoters can be substituted for the E4 promoter. Because E4 gene products are essential for Ad replication, placing their expression under the control of a tumor-specific promoter should restrict replication of the vector to tumor cells in which the promoter is activated.

- 5 Another strategy for restricting replication of ADP-expressing Ad vectors to neoplastic cells is exemplified by the KD1 (SEQ ID NO:1), KD2 (SEQ ID NO:13) and KD3 (SEQ ID NO:2) vectors, whose construction and properties are described in the Examples below. This strategy exploits a pre-existing Ad5 mutant in the E1A gene, named *d1101/1107* (Howe et al., *Proc. Natl. Acad. Sci.*, 87:5883-5887, 1990), also referred to herein as *d101/07*,  
10 and which can only grow well in cancer cells. The role of E1A is to drive cells from the G<sub>0</sub> and G<sub>1</sub> phases of the cell cycle into S-phase. This is achieved by two mechanisms, one involving pRB (and family members), and the other involving p300 and the related protein CBP (DePinho, R.A., *Nature* 391:533-536, 1998). One domain in E1A binds members of the pRB family. pRB normally exists in the cell as a complex with the transcription factor E2F-1  
15 and E2F family members (E2F), tethered via E2F to E2F binding sites in promoters of cells expressed in S-phase. Here, pRB acts as a transcriptional co-repressor. E1A binding to pRB relieves this repression, and causes the release of E2F from pRB/E2F complexes. Free E2F then activates promoters of genes expressed in S-phase, e.g. thymidine kinase, ribonucleotide reductase, etc. Another domain in E1A binds the p300/CBP transcription adaptor protein  
20 complex. p300/CBP is a transcriptional co-activator that binds many different transcription factors and accordingly is targeted to promoters. p300/CBP has intrinsic histone acetyltransferase activity. E1A binding to p300/CBP is believed to inhibit this histone acetyltransferase activity, allowing acetylation of histones and repression of transcription (Chakravarti et al., *Cell* 96:393-403, 1999; Hamamori et al., *Cell* 96:405-413, 1999).  
25 Conceivably, some of the genes that are repressed as a result of E1A interacting with p300/CBP to play a role in blocking the cell cycle, although this is not known. Cancer cells are cycling, so they have free E2F and presumably some p300/CBP-regulated genes are repressed. Consistent with these ideas, E1A must bind both p300/CBP and the pRB family in order to transform primary cells to a constitutively cycling state (Howe et al., *supra*). The  
30 mutant *d101/07* lacks both the p300/CBP- and pRB-binding domains and, as expected, it replicates very poorly in non-dividing "normal" cells or serum-starved cancer cells, but well in growing cancer cells. As described below, the growth of the KD1 and KD3 vectors, which contain the *d101/07* E1A mutation, is very much better in dividing cancer cells as compared to non-dividing cells. Because the *d101/07* mutant is completely defective in oncogenic  
35 transformation of rat cells (Howe et al., *supra*), vectors according to the invention that contain



this E1A mutation cannot induce cancer in humans (remote as that may be) through an E1A-dependent mechanism.

The invention also includes vectors overexpressing ADP whose replication is restricted to specific tissues by placing expression of one or more proteins essential for replication under the control of a tissue specific promoter and/or a tumor specific promoter. A number of tissue-specific and/or tumor specific promoters have been described in the art. Non-limiting examples include the surfactant protein B promoter, which is only active in cells containing the TTF1 transcription factor (i.e., type II alveolar cells (Yan et al., *supra*)), as described in U.S. Patent 5,466,596 to Breitman et al., which directs gene expression specifically in cells of endothelial lineage; prostate specific antigen which is expressed in prostate cells (Rodriguez et al., *supra*); human telomerase protein (hTERT) promoter (see, e.g., U.S. Patent No. 6,054,575); and human alpha-lactalbumin gene which is expressed in breast cancer cells (Anderson et al., *Gene Therapy* 6:854-864, 1999). Many other tissue-specific, tumor specific, or tissue-preferred enhancer/promoters have been reported (Miller and Whelan, *Human Gene Therapy* 8:803-815, 1997). As exemplified with the surfactant protein B promoter in Examples 6 and 10, vectors expressing tissue-specific promoters would be expected to show tissue specificity in viral replication, viral spreading, cell lysis, and tumor suppression.

Replication of vectors according to the invention can also be controlled by placing one or more genes essential for vector replication under the control of a promoter that is activated by an exogenous inducing agent, such as metals, hormones, antibiotics, and temperature changes. Examples of such inducible promoters include but are not limited to metallothionein promoters, the glucocorticoid promoter, the tetracycline response promoter, and heat shock protein (hsp) promoters such as the hsp 65 and 70 promoters.

The invention also provides compositions comprising a recombinant vector that overexpresses ADP in an amount effective for promoting death of neoplastic cells and a method comprising administering a therapeutically effective amount of the vector to a neoplastic cell in a patient. It is believed the compositions and methods of the present invention are useful for killing neoplastic cells of any origin and include neoplastic cells comprising tumors as well as metastatic neoplastic cells.

It is also contemplated that ADP-expressing viral vectors can be administered to neoplastic cells along with a replication-defective virus that expresses an anti-cancer gene product. For example, many replication-defective E1' Ad vectors for use in cancer therapy are well characterized. A limitation of replication-defective vectors is that they only synthesize the therapeutic protein in the cell they initially infect, they cannot spread to other cells. Also, since the genome does not replicate, transcription can only occur from the input

genomes, and this could be as low as one copy per cell. In contrast, the genome of replication-competent Ad vectors are amplified by about  $10^4$  in the cell that was initially infected, providing more templates for transcription. More amplification is achieved as the vector spreads to other cells. By combining replication-defective viral vectors expressing an anti-cancer gene product with replication-competent viral vectors described herein, it is expected that the result will be template amplification and rapid spread of both vectors to surrounding cells. For example, with Ad-based vectors, the burst size for each vector should be large,  $\sim 10^4$  PFU/cell, so the probability of co-infection of surrounding cells by both vectors will be high. Thus, both the replication-competent and replication-defective vectors should spread simultaneously through the tumor, providing even more effective anti-cancer therapy.

As an alternative method of delivering an anti-cancer gene product with an ADP overexpressing Ad vector, the anti-cancer gene can be engineered into any of the ADP overexpressing replication-competent vectors described herein, in order to provide both the ADP and the anti-cancer function in a single vector. The anti-cancer gene can be engineered into any appropriate location of the vector, as can be easily determined by the skilled artisan. For example, the anti-cancer gene can be engineered into the E3 region.

Expression of the anti-cancer gene product encoded by the replication-defective vector can be under the control of either constitutive, inducible or cell-type specific promoters. The anti-cancer gene product can be any substance that promotes death of a neoplastic cell. The term "gene product" as used herein refers to any biological product or products produced as a result of the biochemical reactions that occur under the control of a gene. The gene product can be, for example, an RNA molecule, a peptide, a protein, or a product produced under the control of an enzyme or other molecule that is the initial product of the gene, i.e., a metabolic product. For example, a gene can first control the synthesis of an RNA molecule which is translated by the action of ribosomes into a prodrug converting enzyme which converts a nontoxic prodrug administered to a cancer patient to a cell-killing agent; the RNA molecule, enzyme, and the cell-killing agent generated by the enzyme are all gene products as the term is used here. Examples of anti-cancer gene products include but are not limited to cell-killing agents such as apoptosis-promoting agents and toxins; prodrug converting enzymes; angiogenesis inhibitors; and immunoregulatory molecules and antigens capable of stimulating an immune response, humoral and/or cellular, against the neoplastic cell.

Apoptosis-promoting agents include but are not limited to the pro-apoptotic members of the BCL-2 family such as BAX, BAD, BID and BIK, as well as antisense molecules which block expression of anti-apoptotic members of the BCL-2 family. Examples of immunoregulatory molecules are cytokines such as tumor necrosis factor, Fas/Apo1/CD95

ligand, tumor necrosis factor related apoptosis inducing ligand, interleukins, macrophage activating factor and interferon  $\gamma$ . Angiogenesis inhibitors include but are not limited to endostatin and angiostatin. Toxins include but are not limited to tumor necrosis factor, lymphotoxin, the plant toxin ricin, which is not toxic to humans due to the lack of ricin  
5 receptors in animal cells, and the toxic subunit of bacterial toxins. Examples of pro-drug converting enzymes and pro-drug combinations are described in WO 96/40238 and include thymidine kinase and acyclovir or gancyclovir; and bacterial cytosine deaminase and 5-fluorocytosine.

The therapeutic or pharmaceutical compositions of the present invention can be  
10 administered by any suitable route known in the art including for example by direct injection into a tumor or by other injection routes such as intravenous, subcutaneous, intramuscular, transdermal, intrathecal and intracerebral. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation. For treating tissues in the central nervous system, administration can be by injection or  
15 infusion into the cerebrospinal fluid (CSF). When it is intended that a recombinant vector of the invention be administered to cells in the central nervous system, administration can be with one or more agents capable of promoting penetration of the vector across the blood-brain barrier. Preferably, vectors of the invention are administered with a carrier such as liposomes or polymers containing a targeting moiety to limit delivery of the vector to targeted cells.  
20 Examples of targeting moieties include but are not limited to antibodies, ligands or receptors to specific cell surface molecules.

Compositions according to the invention can be employed in the form of pharmaceutical preparations. Such preparations are made in a manner well known in the pharmaceutical art. One preferred preparation utilizes a vehicle of physiological saline  
25 solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection.  
30 The primary solvent can be aqueous or alternatively non-aqueous.

The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or  
35 penetration across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage

or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

It is also contemplated that certain formulations containing ADP-expressing vectors are to be administered orally. Such formulations are preferably encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Such calculations can be made without undue experimentation by one skilled in the art. Exact dosages are determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration. Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

The invention also contemplates passively immunizing patients who have been treated with a viral vector overexpressing ADP. Passive immunization can include administering to the patient antiserum raised against the viral vector, or gamma-globulin or vector-specific purified polyclonal or monoclonal antibodies isolated from the antiserum. Preferably, the patient is passively immunized after a time period sufficient for the viral vector to replicate in and spread through the tumor.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the

art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

5

## Example 1

This example illustrates the construction and characterization of the KD1 and KD3 anti-cancer vectors.

- To construct KD1, the inventors deleted the entire E3 region of a unique plasmid, leaving behind only a unique *PacI* site for cloning. The starting plasmid was pCRII, purchased from Invitrogen, containing the Ad5 BamHIA fragment having a deletion of all the E3 genes; the E3 deletion is identical to that for KD1 and GZ3, the sequences of which are given in SEQ ID NO:1 and SEQ ID NO:4, respectively. The ADP gene from Ad5 was cloned into the *PacI* site, then built into the E3 region of the genome of the Ad5 E1A mutant named *dI01/07*. This was done by co-transfecting into human embryonic kidney 293 cells the
- 15 aforementioned BamHIA fragment containing the ADP gene together with the overlapping EcoRIA restriction fragment obtained from *dI01/07*. Complete viral genomes are formed within the cell by overlap recombination between the Ad sequences in the BamHIA fragment in the plasmid and the EcoRIA fragment. KD3 was constructed in the same way except the E3 gene for the 12.5K protein was retained in the starting plasmid. A vector named KD2,
- 20 which marginally overexpress ADP, was also prepared. Plaques of each recombinant Ad were picked, screened, purified, expanded into CsCl-banded stocks, sequenced, titrated, and characterized. GZ1 and GZ3 are Ad vectors that are identical to KD1 and KD3, respectively, except that GZ1 and GZ3 have wild-type E1A sequences as found in Ad5 or in the Ad5 mutant *dI309*. GZ1 and GZ3 were constructed as described for KD1 and KD3 except that the
- 25 EcoRIA fragment of Ad5 was used for GZ1 and GZ3.

- KD1 and KD3 were characterized in cell culture by infecting the human A549 lung carcinoma cell line with high titer ( $1.8 \times 10^{10}$  plaque forming units [PFU] per ml) virus stocks of one of these recombinant vectors, or with one of the control viruses *dI01/07*, *dI309*, *dI327*, and Ad5 (wt). Fifty PFU per cell were used for each virus. The descriptions of these viruses
- 30 as well as some other viruses used in these examples are presented in Table 1.

Table 1: Description of mutations in viruses:

us	RNA			REGION	
	E1	VA	E3	E4	
101/1107	<i>d1101</i> : deletion of Ad5 bp 569-634 <i>d1107</i> : deletion of Ad5 bp 890-928	From <i>d1309</i> deletion of Ad5 bp 10594-10595	From <i>d1309</i> deletion of Ad5 bp 28597-28602; deletion-substitution Ad5 bp 3005-30750, insert 642 bp DNA of unknown origin	wild type	
11	<i>d1101</i> : deletion of Ad5 bp 569-634 <i>d1107</i> : deletion of Ad5 bp 890-928	From <i>d1309</i> deletion of Ad5 bp 10594-10595	deletion of Ad5 bp 27858-2760, TAA inserted; deletion of Ad5 bp 27982-28134; deletion of Ad5 bp 28395-29397, insert CCTTAATTAAA; deletion of Ad5 bp 29783-30883, insert TTAATTAAAGG	wild type	
12	<i>d1101</i> : deletion of Ad5 bp 569-634 <i>d1107</i> : deletion of Ad5 bp 890-928	From <i>d1309</i> deletion of Ad5 bp 10594-10595	<i>d1309</i> background, gp19K mutated deletion of Ad5 bp 28597-28602; deletion-substitution Ad5 bp 3005-30750, insert 642 bp DNA of unknown origin; deletion of Ad5 bp 28788-28789, insert TTAATTAA	wild type	
13	<i>d1101</i> : deletion of Ad5 bp 569-634 <i>d1107</i> : deletion of Ad5 bp 890-928	From <i>d1309</i> deletion of Ad5 bp 10594-10595	deletion of Ad5 bp 28598-29397; deletion of Ad5 bp 29783-30469	wild type	
14	wt	wild type	deletion of Ad5 bp 27858-2760, TAA inserted; deletion of Ad5 bp 27982-28134; deletion of Ad5 bp 28395-29397, insert CCTTAATTAAA; deletion of Ad5 bp 29783-30883, insert TTAATTAAAGG	wild type	

;	wild type	wild type	deletion of Ad5 bp	wild type
01/1107-	<i>dl1101</i> : deletion of Ad5 bp 569-634 <i>dl1107</i> : deletion of Ad5 bp 890-928	From <i>dl309</i> deletion of Ad5 bp 10594-10595	From <i>dl309</i> deletion of Ad5 bp 28597-28602; deletion-substitution Ad5 bp 3005-30750, insert 642 bp DNA of unknown origin	E4 promoter deletion-substitution: deletion of Ad5 bp 35623-35775, insert SP-B 500 promoter flanked by Bst1 1071 sites
[SPB	<i>dl1101</i> : deletion of Ad5 bp 569-634 <i>dl1107</i> : deletion of Ad5 bp 890-928	From <i>dl309</i> deletion of Ad5 bp 10594-10595	deletion of Ad5 bp 27848-2760, TAA inserted; deletion of Ad5 bp 27982-28134; deletion of Ad5 bp 28395-29397, insert CCTTAATTAAA; deletion of Ad5 bp 29783-30883, insert TTAATTAAAGG	E4 promoter deletion-substitution: deletion of Ad5 bp 35623-35775, insert SP-B 500 promoter flanked by Bst1 1071 sites
i-SPB	<i>dl1101</i> : deletion of Ad5 bp 569-634 <i>dl1107</i> : deletion of Ad5 bp 890-928	From <i>dl309</i> deletion of Ad5 bp 10594-10595	deletion of Ad5 bp 28598-29397; deletion of Ad5 bp 29783-30469	E4 promoter deletion-substitution: deletion of Ad5 bp 35623-35775, insert SP-B 500 promoter flanked by Bst1 1071 sites

Using a polymerase chain reaction (PCR)-based protocol, an in-frame stop codon was introduced into the gene for the E3-gp19K protein in the E3 region of the Ad5 mutant d309 (Jones and Shenk, *Cell* 17:683-689, 1979). The mutagenesis was conducted using a *SunI*-Bst1107I fragment, nucleotides 28,390 to 29,012 in the Ad5 genome, which was then substituted for the equivalent fragment in d309. d309 is the parent for KD1 and KD3. In turn, the Ad5 mutant named d309 is the parent of d309, i.e. d309 is identical to d309 except that d309 does not have the E1A mutation. Both d309 and d309 have deletions of the genes for the E3 RID $\alpha$ , RID $\beta$  and 14.7K proteins but retain the gene for ADP. The Ad5 mutant d327 has wild-type E1A, it lacks the gene for ADP, and it lacks all other E3 genes except the one for the 12.5K protein.

At 24 and 36 hours post-infection (h p.i.), proteins were extracted from the A549 cells and analyzed for ADP by immunoblot using a rabbit antiserum against ADP (Tollefson et al., *J. Virol.* 66:3633-3642, 1992). The results are shown in Figure 2. Much more ADP was detected at 24 and 36 h p.i. in KD1- and KD3-infected cells than in cells infected with d309. Also, much more ADP was synthesized by GZ1 and GZ3 than d309 or the other viruses. Most importantly, KD1, KD3, GZ1, and GZ3 expressed much more ADP at 24 h p.i. than did d309 or d309 (Fig. 2). This result is consistent with an observation discussed below that the cells infected with KD1, KD3, GZ1, or GZ3 lyse faster, and that these viruses spread from cell to cell faster than d309 or d309. It is noteworthy that KD1, KD3, GZ1, and GZ3 express much more ADP at 24 and 36 h p.i. than the Ad5 mutant d1520 (Fig. 2); d1520 is the original name given to ONYX-015 (Heise et al., *Nature Medicine* 3:639-645, 1997). As expected, no ADP was detected in cells infected with pm734.1 (Fig. 2), a mutant that lacks amino acids 1 to 48 in ADP (Tollefson et al., *J. Virol.* 70:2296-2306, 1996). Expression of the E1A proteins by d309, KD1, KD2, and KD3 was slightly less than by Ad5, d309, or d327, and as expected from the d309 deletion, the proteins were smaller (Fig. 3A). d327 is isogenic with d324 (Thimmappaya et al., 1982 *Cell* 31:543-51, 1983), and it lacks the gene for ADP and all other E3 proteins except the 12.5K protein.

The amount of ADP detected in the KD1 and KD3 infected cells is significantly higher than the amount detected in the d309 infected cells (Fig. 2). If one takes into consideration the fact that the viruses with the E1A mutation replicate somewhat slower, as evidenced in by the delayed appearance of the late proteins (Fig. 3B), it is clear that KD1 and KD3 express much more ADP per viral genome present in the cell than d309. This finding is supported by the fact that when A549 cells are coinfecting with a virus containing the E1A mutation and d327, which lacks ADP but has wild-type E1A, the replication rates of the E1A mutant viruses speed up, as indicated by earlier appearance of late proteins (compare Figs. 3B



and 3D). Thus, *dI327* complements the E1A mutation. In conclusion, these experiments demonstrate that ADP is dramatically overexpressed by KD1, KD3, GZ1, and GZ3. ADP is marginally overexpressed by KD2 (not shown).

#### Example 2

5 This example illustrates that KD1 and KD3 lyse cells more rapidly and spread from cell-to-cell faster than other adenoviruses.

The ability of KD1 and KD3 to lyse cells was examined by a trypan blue exclusion cell viability assay which was performed essentially as described by Tollefson et al., *J. Virol.* 70:2296-2306, 1996. In brief, A549 cells were mock-infected or infected with 20 PFU/cell of  
10 KD1, KD3, *dI01/07*, *dI327* or *dI309*. At various days p.i., the number of viable cells was determined using a hemocytometer (600 to 1000 cells were counted per time point) and the results are shown in Fig. 4.

Only 25% of the KD1-infected cells and 9% of the KD3-infected cells were alive at 5 days p.i. as compared to 44% of cells infected with *dI01/07*, which has the same E1A  
15 mutation as KD1 and KD3. The KD1 and KD3 vectors also lysed cells faster than *dI309*, which has a wild-type E1A region. When infected with *dI327* (ADP, E1A<sup>-</sup>), 94% of the cells were alive after 5 days. When cell lysis was estimated by release of lactate dehydrogenase, KD1 and KD3 once again lysed cells faster than *dI01/07* and *dI309*, and *dI327* caused little cell lysis (data not shown). Thus, ADP is required for efficient cell lysis, and over-expression  
20 of ADP increases the rate of cell lysis.

As another means to measure cell lysis and to examine virus replication in cancer cells, separate groups of A549 cells were infected with 20 PFU/cell of KD1, KD3, *dI01/07*, or *dI309* and the amount of intracellular and extracellular virus was determined by plaque assay on A549 cells. At 2 days p.i., the total amount of virus formed in each group was similar, 2-4  
25  $\times 10^8$  PFU/ml, indicating that replication of all the viruses is similar. However, when the ratio of extracellular to intracellular virus was calculated, the value for KD1 and KD3 was 2-3 logs higher than for Ad5, *dI309*, or *dI01/07* (data not shown). Thus, virus is released much more rapidly from cells infected with KD1 and KD3, which overexpress ADP, than with viruses expressing wild-type amounts of ADP.

30 The ability of KD1 and KD3 to spread from cell-to-cell was measured in a "cell spreading" assay. In this assay monolayers of A549 cells in a 48 well culture dish were mock-infected or infected with  $10^{-3}$ ,  $10^{-2}$ ,  $10^{-1}$ ,  $10^0$ , or 10 PFU/cell of *dI327*, *dI309*, Ad5, *dI01/07*, KD1 or KD3. At low PFU/cell, the viruses must go through two or three rounds of replication in order to infect every cell in the monolayer. At 1.0 and 10 PFU/cell, the  
35 monolayer should be destroyed by the virus that initially infected the cells. To assess the

amount of spread in the monolayers by 7 days p.i., crystal violet, which stains live cells but not dead cells, was added to the monolayers. The results are shown in Fig. 5.

Remarkably, at 7 days p.i., the monolayer was virtually eliminated by KD1 and KD3 at  $10^{-3}$  PFU/cell, whereas 1.0 PFU/cell was required with *d*/01/07, *d*/309 and Ad5. This result attests to the potency of ADP in mediating cell lysis and virus spread in A549 cells. KD1 and KD3 are also more effective than *d*/01/07 in killing other types of human cancer cell lines (most purchased from the American Type Culture Collection [ATCC]) as determined in this cell spreading assay. KD1 and/or KD3 killed HeLa (cervical carcinoma), DU145 (prostate), and pC3 (prostate) cells at  $10^{-2}$  PFU/cell, ME-180 (cervix) and Hep3B (liver) at  $10^{-1}$  PFU/cell, and U118 (glioblastoma) and U373 (glioblastoma) at 10 PFU/cell. From 10- to 100-fold more *d*/01/07 was required to kill these cells (data not shown). These results indicate that KD1 and KD3 may be effective against many types of cancer.

An important aspect of the finding that ADP overexpressing vectors lyse cells at very low multiplicities of infection is that the multiplicity of infection in human tumors is likely to be low at sites distal to the sight of vector injection or distal to blood vessels that carry the vector to the tumor. Thus, ADP overexpressing vectors have an advantage over vectors that express less ADP or no ADP at all.

### Example 3

This example illustrates that KD1 and KD3 replicate poorly in non-growing non-cancerous cells. The replication phenotype of KD1 and KD3 was evaluated using "normal" HEL-299 human fibroblast cells, either growing in 10% serum or rendered quiescent using 0.1% serum. All Ads should replicate well in growing cells, but viruses with the *d*/01/07 E1A mutation should do poorly in quiescent cells because E1A is required to drive them out of G<sub>0</sub>. *d*/309, which has wild-type E1A, should replicate well in both growing and growth-arrested cells.

Cells were infected with 100 PFU/cell of KD1, KD3, *d*/01/07, or *d*/309. At different days p.i., virus was extracted and titered. In 10% serum, KD1, KD3, and *d*/01/07 replicated well, reaching titers of  $10^6$ - $10^7$  PFU/ml, only slightly less than *d*/309 (Fig. 6). However, in quiescent cells, replication of KD1, KD3, and *d*/01/07 was 1.5-2 logs lower than in growing cells, ranging from  $10^4$  to  $2 \times 10^5$  PFU/ml. The titer of *d*/309 reached  $10^7$  PFU/ml, nearly the level achieved in growing cells. At 10 days p.i., quiescent HEL-299 cell monolayers infected with 100 PFU/cell of KD1, KD3, or *d*/01/07 were intact, whereas those infected with *d*/309 or *d*/327, which have wild-type E1A, showed strong typical Ad cytopathic effect indicative of cell death (data not shown). Thus, replication of KD1 and KD3 is severely restricted to growing cell lines.

- The restriction associated with the *dI01/07* E1A mutation was also tested in primary human cells (purchased from Clonetics) growing as monolayers. Bronchial epithelial cells (Fig. 7) and small airway epithelial cells were not killed by 10 PFU/cell of KD1, KD3, or *dI01/07* at 5 days p.i., whereas they were killed by 10 PFU/cell of *dI309* or *dI327* (data not shown). Lung endothelial cells also were not killed after 10 days by KD1, KD3, or *dI01/07* at 10 PFU/cell, but they were killed by 1 PFU/cell of *dI309*. These monolayers were subconfluent when initially infected, then grew to confluency. The exciting result here is that although these primary cells were growing, they did not support replication in this time frame and were not killed by KD1 or KD3. Thus, it is believed these vectors will be restricted to cancerous cells, and will have little to no effect on cells such as basal cells that are normally dividing in the body. In addition, it is unlikely that KD1 and KD3 will affect dividing leukocytes because such cells are poorly infected by Ad.
- In summary, the above experiments demonstrate that KD1 and KD3 lyse cancer cells, spread from cell-to-cell rapidly, and replicate poorly in quiescent and non-cancerous cells. These properties should make them useful in anti-cancer therapy.

#### Example 4

This example illustrates that KD1 and KD3 inhibit the growth of human tumors in an animal model.

- We could not evaluate mouse or rat tumors in normal mice or rats because they are totally non-permissive. Human cancer cell lines growing in nude mice have been used by Onyx Pharmaceuticals (Richmond, CA) to evaluate the efficacy of ONYX-015, an Ad vector lacking expression of the E1B 55 kDa protein (Heise et al., *Nature Med.* 3:639-645, 1997). We have found that A549 cells, which were used in many of our cell culture studies, form excellent rapidly growing solid tumors when injected subcutaneously into nude mice. The average tumor reaches ca. 500  $\mu$ l in four weeks, and is encapsulated, vascularized, and attached to the mouse skin (usually) or muscle.

- Nude mice were inoculated into each hind flank with  $2 \times 10^7$  A549 cells. After 1 week tumors had formed, ranging in size from about 20  $\mu$ l to 50  $\mu$ l. Individual tumors were injected three days later, and at subsequent weeks for 4 weeks (total of 5 injections), with 50  $\mu$ l of buffer or 50  $\mu$ l of buffer containing  $5 \times 10^7$  PFU of *dI309*, *dI01/07*, KD1, KD3, or *pm734.1*, with a total virus dose per tumor of  $3 \times 10^8$  PFU. The mutant *pm734.1* lacks ADP activity due to two nonsense mutations in the gene for ADP, but all other Ad proteins are expected to be synthesized at wild-type levels (Tollefson et al., *J. Virol.* 70:2296-2306, 1996). The efficacy of each virus (or buffer) was tested on six tumors. At weekly intervals, the length (L) and width (W) of tumors were measured using a Mitutoyo digital caliper. Tumor

volumes were calculated by multiplying  $L \times W \times W/2$ . This value was divided by the tumor volume at the time of the initial virus injection, the fold-increase in tumor growth was calculated, and the average for the six tumors was graphed.

As shown in Fig. 8A, tumors that received buffer continued to grow, increasing about 14-fold by 5 weeks. In contrast, tumors injected with *d/309*, which expresses normal amounts of ADP and lacks the E3 RID and 14.7K and proteins, only grew about 2.5-fold by 5 weeks. With *pm734.1*, which lacks ADP, the tumors grew as well as those that received buffer. Thus, *d/309* markedly decreases the rate of tumor growth, and ADP is required for this decrease. Tumors inoculated with *d/01/07* grew about 8-fold over 5 weeks. Since *d/01/07* is identical to *d/309* except for the E1A mutation, this result indicates that the E1A mutation significantly reduces the ability of Ad to prevent growth of the tumors. This effect is probably due to a reduction in virus replication in the tumors resulting in lower ADP expression, but it could also reflect other properties of E1A in the tumor cells, e.g. the inability of the mutant E1A proteins to induce apoptosis. Most importantly, tumors inoculated with KD1 or KD3 only grew about 2.5-fold. Thus, the overexpression of ADP by KD1 and KD3 allows KD1 and KD3 to reduce tumor growth to a rate markedly slower than *d/01/07* (their parental control virus), and even to a rate similar to that of *d/309*.

The finding that KD1 and KD3 are as effective as wild-type Ad (i.e. *d/309*) in reducing the rate of A549 tumor growth is highly significant in the context of cancer treatment, inasmuch as KD1 and KD3 are restricted to cancer cells whereas wild-type Ad does not have such a restriction.

The tumors in Fig. 8A received five injections of vectors, but only one dose of vector, in this case  $5 \times 10^8$  of each of KD3 or GZ3, is sufficient to significantly reduce the rate of A549 tumor growth (Fig. 8B).

We have also found that KD1 and KD3 reduce the rate of growth in nude mice of a human liver cancer cell line, Hep3B cells. These cells form rapidly growing tumors that are highly vascularized. Nude mice were inoculated into each hind flank with  $1 \times 10^7$  of Hep3B cells. After tumors reached about 100  $\mu$ l, they were injected twice per week for 3 weeks with 50  $\mu$ l of buffer or  $5 \times 10^7$  PFU of KD1, KD3, or *d/309*. There were typically 8-10 tumors per test virus. The tumor sizes were measured and the fold increase in size at 0 to 3.5 following the initial virus injection was graphed as described above for the A549 tumors. Tumors that received buffer alone grew 9-fold over 3 weeks and were projected to grow about 12-fold over 3.5 weeks (after 3 weeks the mice had to be sacrificed because the tumors were becoming too large) (Fig. 9). Tumors that received KD1 or KD3 grew about 4-fold, establishing that KD1 and KD3 reduce the growth of Hep3B tumors in nude mice. Tumors

that were injected with *dI309* grew 2-fold (Fig. 9). The finding that KD1 and KD3 were somewhat less effective than *dI309* is probably due to the fact that they do not grow as well as *dI309* in Hep3B cells, as indicated by a cell spread assay in culture (data not shown). In any case, the important points are that KD1 and KD3 are effective against the Hep3B tumors, and that they contain the E1A mutation that limits their replication to cancer cells.

These results point to the potency of ADP as an anti-tumor agent when expressed in an Ad vector. It is highly probable that KD1 and KD3 will provide significant clinical benefit when used to infect tumors growing in humans.

#### Example 5

This example illustrates the use of replication-defective Ad vectors in combination with KD1 or KD3.

It is well established that replication-competent (RC) viruses complement replication-defective (RD) mutants. That is, when the same cell is infected, the competent virus will supply the protein(s) that cannot be made from the mutant genome, and both viruses will grow. To test the ability of KD1 and KD3 to complement RD viruses, two RD vectors expressing  $\beta$ -galactosidase were constructed. The first, named Ad- $\beta$ -gal, has a cDNA encoding  $\beta$ -gal under the control of the Rous Sarcoma Virus promoter substituted for the deleted E1 region. Ad- $\beta$ -gal also has the E3 region deleted, including the gene for ADP. The second, named Ad- $\beta$ -gal/FasL is identical to Ad- $\beta$ -gal, except that it also expresses murine FasL from the human cytomegalovirus promoter/enhancer. These vectors were constructed by overlap recombination in human 293 cells that constitutively express the Ad E1A and E1B genes and complement replication of the E1-minus vectors.

These RD vectors should infect and express  $\beta$ -gal in A549 cells, but should not replicate because the E1A proteins are lacking. However, the vectors should replicate when cells are co-infected with RC Ads. To prove this, A549 cells were infected with 10 PFU/cell of Ad- $\beta$ -gal alone, or with 10 PFU/cell of Ad- $\beta$ -gal plus 10 PFU/cell of KD1, KD3, *dI01/07*, *dI309*, or *dI327*. At 2 days p.i., virus was extracted and Ad- $\beta$ -gal titers determined by  $\beta$ -gal expression in A549 cells. The yields are shown in Table 2 below.

Table 2

Virus	Yield (blue plaques per ml)
Ad- $\beta$ -gal	$1 \times 10^2$
Ad- $\beta$ -gal + KD1	$2 \times 10^5$
Ad- $\beta$ -gal + KD3	$3 \times 10^5$
Ad- $\beta$ -gal + <i>dI01/07</i>	$4 \times 10^4$
Ad- $\beta$ -gal + <i>dI309</i>	$3 \times 10^5$
Ad- $\beta$ -gal + <i>dI327</i>	$3.0 \times 10^5$

The data in Table 2 indicate that the complementing viruses increased the yield of Ad- $\beta$ -gal by about  $10^3$ .

- 5 A key feature of KD1 and KD3 is that they spread from cell-to-cell faster than other Ads. Accordingly, they should complement the spread of Ad- $\beta$ -gal. To test this, an infectious center assay was conducted. A549 cells were infected with Ad- $\beta$ -gal plus KD1, KD3, or *dI01/07*. After 2 h, cells were collected, diluted, and seeded onto monolayers of fresh A549 cells. After 4 days, the cells were stained with X-gal and the results are shown in
- 10 Fig. 10.

- With Ad- $\beta$ -gal alone, only the originally infected cell (before seeding) should be stained, and the vector should not spread to other cells on the seeded monolayer. This was indeed the case. In monolayers seeded with A549 cells infected with Ad- $\beta$ -gal alone (dish shown in the top left of Fig. 10A) contained a number of individual blue cells (not visible in
- 15 the print); examples are shown in the enlarged view Fig. 10B. However, when the monolayers were seeded with A549 cells coinfecting with Ad- $\beta$ -gal and KD1 or KD3, there were numerous "comets" of blue cells (Fig. 10A). Each comet represents Ad- $\beta$ -gal which has spread from one initially-infected cell. Most of the cells within a comet were stained with X-gal (Fig. 10C). Comets were also observed with *dI01/07*, but not to the extent of KD1 and
- 20 KD3 (Fig. 10A). With *dI327* (ADP<sup>+</sup>), there was little spread from the originally infected cell (data not shown). In summary, KD1 and KD3 not only complement the replication of Ad- $\beta$ -gal, they also enhance its rapid spread.

It is expected that KD1 and KD3 will also complement and enhance the spread of RD vectors expressing anti-cancer therapeutic gene products, and this expectation can be readily

verified using the Ad- $\beta$ -gal/FasL in replication and infectious center assays as described above.

KD1 and KD3 not only complement the replication of RD vectors in cell culture, they also do so in Hep3B tumors growing in the hind flanks of nude mice. The RD vector used was AdLuc, an Ad that lacks the E1 and E3 regions, and has inserted into the E1 region an expression cassette where the firefly luciferase gene is expressed from the Rous Sarcoma Virus promoter (Harrod et al., *Human Gene Therapy* 9:1885-1898, 1998). The Hep3B tumors were injected with  $1 \times 10^7$  PFU of AdLuc plus buffer, or  $1 \times 10^7$  PFU of AdLuc plus  $5 \times 10^7$  PFU of KD1, KD3, *dI01/07*, or *dI309*. After 2 weeks, mice were sacrificed and tumors excised. Proteins were extracted from the tumors and luciferase activity determined using a luminometer. The luciferase counts per tumor were 6,800 for AdLuc plus buffer, 113,500 for KD1, and 146,900 for KD3 (Fig. 11). Thus, KD3 and KD1 respectively caused a 22-fold and 17-fold increase in luciferase activity. This increase could be due to elevated synthesis of luciferase in cells that were initially coinfecting the AdLuc and KD1 or KD3, and it could also be due to spread of AdLuc from cell to cell in the tumor as mediated by KD1 or KD3.

In summary, infecting a tumor with a replication-competent ADP-overexpressing vector according to the invention together with a RD vector expressing an anti-cancer gene product should greatly increase the amount of anti-cancer protein synthesized in the tumor thereby increasing the ability of the replication-defective vector to promote destruction of the tumor.

#### Example 6

This example illustrates the construction and characterization of a recombinant Ad vector according to the invention which is replication-restricted to cancerous type II alveolar cells.

As demonstrated above, the *dI01/07* mutation in KD1 and KD3 limits growth of these vectors to cancer cells. To further restrict their replication phenotype, the E4 promoter in each virus was deleted and replaced by the surfactant protein B (SPB) promoter to produce vectors named KD1-SPB (SEQ ID NO:14), KD3-SPB (SEQ ID NO:15), and *dI01/07*-SPB (SEQ ID NO:16). The SPB promoter is only active in cells containing the TTF1 transcription factor, which has thus far been found primarily in type II alveolar cells of the human lung (Lazzaro et al., *Development* 113:1093-1104, 1991). Thus, KD1-SPB, KD3-SPB, and *dI01/07*-SPB should be severely restricted to cancerous type II alveolar cells of the human lung. Many lung cancers are of this type.

The KD1-SPB and KD3-SPB vectors were prepared as follows. The E4 promoter is located at the right end of the Ad genome (Fig. 1). Using a pCRIT-based plasmid (Invitrogen)

containing the Ad5 DNA sequences from the BamHI site (59 map units) to the right hand end of the genome, and using a PCR-based protocol, nearly all the transcription factor binding sites were deleted from the E4 promoter Ad5 base pairs 35,623 to 35,775 and replaced with a 500 base pair fragment containing the SPB promoter (Yan et al., *J. Biol. Chem.* 270:24852-24857, 1995). The final plasmids contain the E4-SPB substitution in the E4 region and the *d*/01/07, KD1, or KD3 versions of the E3 region, respectively, for the viruses *d*/01/07-SPB, KD1-SPB, and KD3-SPB. These plasmids were co-transfected into 293 cells with a fragment containing the left portion of the genome of *d*/01/07, and plaques were allowed to develop. Plaques were screened for the expected features, purified, then expanded into a stock.

10 The A549-TTF1 cell line was developed in order to test the prediction that replication of *d*/01/07-SPB, KD1-SPB, and KD3-SPB would be restricted to cancerous cells expressing the TTF1 transcription factor. These cells were co-transfected with two plasmids, one in which TTF1 is expressed from the CMV promoter, and the other coding for resistance to neomycin. Resistant clones were isolated and shown to express TTF1 activity as determined by transient transfection with a plasmid expressing chloramphenicol acetyltransferase from the TTF1-requiring surfactant protein C promoter.

15 KD1-SPB and KD1 were subjected to a standard plaque development assay on A549-TTF1 cells and parental A549 cells. The results are shown in Fig. 12. With KD1-SPB on A549 cells, plaques were not visible after 8 days, only about 4% of the final number of plaques were seen after 10 days, and about 50% of final plaques were seen after 12 days. With KD1-SPB on A549-TTF1 cells, plaques were visible after 6 days, and about 60% of plaques were seen after 10 days. Thus, as expected, KD1-SPB grew significantly faster on the cells containing TTF1. KD1 formed plaques more quickly than KD1-SPB on both A549 and A549-TTF1 cells, indicating that the E4 promoter-SPB substitution is not as effective the wild-type E4 promoter in inducing Ad replication. However, this difference between KD1-SPB and KD1 on A549-TTF1 cells is tolerable, with KD1-SPB delayed only about 1 day. Curiously, the final titer obtained for all virus stocks by day 16 was similar, indicating that A549 cells may contain a very small amount of endogenous TTF1 activity. It is predicted that KD3-SPB and *d*/01/07-SPB will behave similarly to KD1-SPB when grown in A549-TTF1 cells and A549 cells.

20 The restriction of KD1-SPB to cells containing TTF1 was further examined in a cell spread assay using H441 cells, a TTF1-expressing human pulmonary adenocarcinoma cell line (Yan et al., *supra*), and Hep3B cells, a liver cancer cell line not expected to express TTF1. Culture dish wells containing H441 or Hep3B cells were infected with KD1-SPB or KD1 at multiplicities ranging from 10 to  $10^4$  PFU/cell. The H441 and Hep3B cells were



stained with crystal violet at 5 days and 8 days p.i., respectively. KD1-SPB and KD1 grew and spread equally well on H441 cells, causing destruction of the monolayer at  $10^{-1}$  PFU per cell (Fig. 13). (Some of the H441 monolayer has peeled off in the well with KD1-SPB at  $10^{-2}$  PFU per cell, and in the wells with KD1 and KD1-SPB at  $10^{-4}$  PFU per cell; this occasionally occurs in cell spread assays, and it does not reflect virus infection). With Hep3B cells, KD1 grew and spread very much better than KD1-SPB, with  $10^{-2}$  PFU per cell of KD1 causing more destruction of the monolayer as 1.0 PFU per cell of KD1-SPB (Fig. 13).

In summary, this example demonstrates that a replication-competent Ad, which replicates well on cells expressing the appropriate transcription factor, can be constructed with a tissue-specific promoter substituted in place of the E4 promoter. This methodology should be applicable to many other tissue specific and cell type specific promoters. One possibility would be a liver-specific promoter. Another possibility would be to use the E2F promoter, or another promoter with E2F sites, inasmuch as that promoter would be active only in cells such as cancer cells that have free E2F. A third possibility would be to use a regulatable promoter, e.g. the synthetic tetracycline response promoter (Massie et al., *J. Virol.* 72:2289-2296, 1998), where the activity of the promoter is controlled by the level of tetracycline or a tetracyclin analog in the patient.

#### Example 7

This example illustrates the construction and characterization of vectors which overexpress ADP and are not replication restricted.

As demonstrated above, the *d/01/07* E1A mutation in KD1 and KD3 is attenuating, inhibiting growth in non-dividing and even in dividing primary human epithelial and endothelial cells. Ads with this mutation are able to replicate well in dividing cancer cells. However, replication of such E1A mutants is not as efficient as, e.g. *d/309* which has a wild-type E1A gene. For instance, the rate of replication of *d/01/07*, as determined by the rate at which plaques develop, is reduced such that *d/01/07* plaques appear one day later than those of *d/309* (data not shown). This delay is due in part to a delay in expression of Ad late genes (see Fig. 3). The idea that the *d/01/07* mutation retards the rate of replication in A549 cells is further supported by the data in Fig. 8A, where *d/01/07* did not prevent tumor growth nearly as well as *d/309*. Despite this negative effect of the *d/01/07* E1A mutation, there are theoretical and practical aspects of having this mutation in the KD1 and KD3 vectors, as has been discussed. Nevertheless, one can easily imagine scenarios (e.g. patients with terminal cancer) where the ability of an Ad vector to destroy the tumor supercedes the requirement that the vector be totally restricted to tumor cells. In such cases, it would be advantageous to have vectors similar to KD1 and KD3, but with the wild-type E1A gene. The rates at which such

vectors express their genes, lyse cells, and spread from cell to cell should be higher than those of KD1 and KD3. Such vectors might cause some damage to non-cancerous cells and tissue, but this is also true for other modes of anti-cancer treatment such as surgery, chemotherapy, and radiation therapy.

- 5 In light of these considerations, vectors named GZ1 and GZ3 have been constructed that are identical to KD1 and KD3, respectively, except they have a wild-type E1A region. These vectors were constructed by overlap recombination in A549 cells. The left hand fragment contained the wild-type E1A region of Ad5, and the right end fragment contained the E3 modifications of KD1 or KD3. Plaques were picked, analyzed for the expected
- 10 genotype, plaque-purified, and expanded into CsCl-banded stocks. The titers of these stocks on A549 cells were  $2.9 \times 10^{10}$  PFU/ml for GZ1 and  $1.6 \times 10^{11}$  PFU/ml for GZ3. Thus, these vectors can be grown into high titer stocks comparable to wild-type Ad. The GZ1 and GZ3 plaques are larger and appear much sooner than the plaques for d/309. Large rapidly-appearing plaques reflect the ability of Ad to lyse cells and spread from cell-to-cell (Tollefson et al., *J. Virol.* 70:2296-2306, 1996; Tollefson et al., *Virology* 220:152-162, 1996), and this
- 15 property, as discussed, is due to the function of ADP.

- The rate of plaque appearance can be quantitated in a plaque development assay (Tollefson et al., *supra*). Here, a typical plaque assay is performed, and the plaques observed on subsequent days of the assay are calculated as a percentage of the number of plaques
- 20 observed at the end of the plaque assay. As shown in Fig. 14, after 4 days of plaque assay on A549 cells, GZ1 and GZ3 had 48% and 34%, respectively, of the final number of plaques, whereas d/309 had only 1%. It is very unusual in Ad plaque assays in A549 cells for plaques to appear after only 4 days. These large plaques reflect the overexpression of ADP. These GZ1 and GZ3 plaques appear sooner than those of KD1 and KD3 (data not shown), no doubt
- 25 because GZ1 and GZ3 replicate faster because they have a wild-type E1A region.

- GZ1 and GZ3 lyse cells and spread from cell to cell much more effectively than d/309. At 6 days p.i. of A549 cells, approximately as much monolayer destruction was observed with GZ1 and GZ3 at  $10^3$  PFU per cell as was observed with d/309 at  $10^3$  PFU per cell (Fig. 15, top panel). This result further underscores the conclusion that overexpression of
- 30 ADP promotes cell lysis and virus spread.

- In theory, GZ1 and GZ3 should be able to replicate not only in tumor cells but also in normal cells. Although they can replicate in normal cells, it is quite possible that GZ1 and GZ3 may be useful as anti-cancer vectors. First, GZ1 and GZ3 could be injected directly into the tumor. Many tumors are self-contained (encapsulated) except for the blood supply. The
- 35 physical barriers of the tumor could minimize dissemination of the virus to other tissues.

- Second, Ads are in general quite benign. Most infections of Ad5 are in infants and result in mild or asymptomatic disease, and are held in check by strong humoral and cellular immunity. Anti-Ad immunity appears to be life-long. GZ1 and GZ3 could be used only in patients who have an intact immune system, and perhaps also with pre-existing anti-Ad immunity. Further, patients could be passively immunized against Ad, using gamma-globulin or even specific purified anti-Ad neutralizing antibodies. Third, considering that Ad5 is a respiratory virus which most efficiently infects lung epithelial cells displaying the specific Ad5 receptor (named CAR) as well as specific integrins (e.g.  $\alpha_v\beta_5$ ), replication-competent vectors derived from Ad5 may not spread efficiently in many non-cancer tissues of the body. In addition, it is believed that versions of GZ1 and GZ3 can be constructed that have the E4 promoter substituted with a tumor-specific, tissue-specific, cell-specific, or synthetic promoter. Such vectors would have the positive features associated with wild-type E1A and ADP, and yet be replication-restricted to tumor tissue and/or to particular cell types.

#### Example 8

- This example illustrates that the combination of KD1, KD3, GZ1, or GZ3 with radiation is more effective in destroying A549 cells, growing in culture or growing as tumors in nude mice, than the vectors alone or radiation alone.

- This was shown in a cell spread assay. A549 cells growing in three 48 well culture dishes were mock-infected or infected with different viruses at multiplicities of infection ranging from  $10^1$  to  $10^4$  PFU per cell as indicated in Fig. 15. One dish was not radiated. A second dish received 600 centigrays (cGy) of radiation at 24 h p.i., and a third dish received 2000 cGy of radiation at the same time. All dishes were stained with crystal violet at 6 days p.i. With the cells that were not radiated (top panel in Fig. 15), KD1 and KD3 caused monolayer destruction at lower multiplicities of infection than their parental control, *d/01/07*. This was also true for GZ1 and GZ3 as compared to their parental control *d/309*. (The paucity of cells in the cells infected with GZ1 or GZ3 at  $10^4$  PFU per cell is an experimental artifact, and is not caused by infection by GZ1 or GZ3). These KD1, KD3, GZ1 and GZ3 results are consistent with earlier results showing that overexpression of ADP leads to increased cell lysis and virus spread.

- With the dish that was infected then radiated with 600 cGy there was markedly increased cell killing and virus spread as compared to the non-radiated cells (compare the bottom panel of Fig. 15 with the top panel). For example, with KD1, KD3, GZ1, and GZ3 there was about the same amount of cell destruction in the radiated wells at  $10^4$  PFU per cell as in the non-radiated wells at  $10^2$  PFU per cell. Similar results were seen with the dish that

received 2000 cGy of radiation (data not shown), and also with dishes that received 600 or 2000 cGy of radiation 24 h prior to infection (data not shown).

The amount of cell destruction was quantitated by extracting the crystal violet from the cells with 33% acetic acid, then measuring the absorbance at 490 nm (data not shown).

- 5 The absorbance with non-radiated mock-infected cells was set at 100% cell viability. With mock-infected cells that received 600 cGy there was a 15% loss in viability (i.e. 15% less crystal violet was extracted). With KD1 at  $10^3$  PFU per cell, the non-radiated cells were 80% viable whereas the cells receiving 600 cGy of radiation were only about 30% viable. Similar differences in viability between radiated and non-radiated cells were seen with KD3, GZ1, 10 and GZ3. These results argue that the combination of radiation plus vector has a synergistic effect on cell lysis and vector spread, rather than an additive effect. If the effect were only additive, then with the KD1 samples at  $10^3$  PFU per cell, the cell viability should have been 65% (15% reduction in viability due to radiation alone, 20% reduction due to KD1 alone). In fact, the cell viability was 30% rather than 65%.

- 15 As mentioned, approximately as much cell lysis and virus spread were observed with 600 cGy as with 2000 cGy. To determine the optimal dose of radiation to synergize with the vectors, an experiment similar to the one described above was conducted with mock-, d101/07-, KD1-, KD3-, d1309, GZ1-, or GZ3-infected A549 cells. The 48 well plates received 0, 150, 300, or 600 cGy of radiation at 24 h p.i. Cells were stained with crystal violet. The results with cells receiving 0 versus 600 cGy of radiation were similar to those in Fig. 15. 20 The crystal violet was extracted from the cells infected with  $10^3$  PFU per cell of the difference viruses. The absorbance of crystal violet was determined, and the percent cell viability was graphed, using the absorbance of the non-radiated mock-infected cells as 100% cell viability. As illustrated in Fig. 16, an approximately linear decrease in cell viability in all 25 wells was obtained with increasing radiation dose, although the slope of the line was more negative with KD1, KD3, GZ1, or GZ3 than with mock, d101/07, or d1309. With KD1, KD3, GZ1, and GZ3, there was much more cell lysis and vector spread with their parental control viruses, and there was synergy between the vectors and radiation. For example, with mock-infected cells, 600 cGy reduced cell viability by about 30% (70% of cells were viable). KD1 30 without radiation reduced cell viability by about 23%. The combination of 600 cGy radiation plus KD1 reduced cell viability to about 85%, more than 53% of which is the sum of radiation alone and KD1 alone. When considering the data in Figs. 15 and 16 together, a dose of about 600 cGy is optimal in this type of cell culture experiment.

- 35 The combination of KD3 or GZ3 with radiation was also examined in the A549 tumor-nude mouse model (see Example 4). A549 cells were injected into the hind flanks of

nude mice, and tumors were allowed to form. When tumors reached approximately 50- $\mu$ l, they were injected with buffer or with  $5 \times 10^8$  PFU of KD3 or GZ3. Eight to ten tumors were injected per test condition. At 1 day p.i., half the mice received 600 cGy of whole body radiation. Tumor size was measured over time, and was plotted as a fold-increase in tumor size versus days p.i. (as described in Example 4). As shown in Fig. 17, the non-radiated buffer-injected tumors grew faster than those injected with KD3 or GZ3. Tumors that received the combination of KD3 and radiation did not grow, and those that received the combination of GZ3 and radiation shrank in size after 14 days. These results indicate that the combination of KD3 plus radiation or GZ3 plus radiation is more effective than either vector alone or radiation alone in reducing the rate of A549 tumor growth in nude mice. It is likely that radiation would increase the effectiveness in treating tumors of KD1 and GZ1, or indeed any other replication-competent or replication-defective Ad vector.

The mechanism by which radiation causes the ADP overexpressing vectors to lyse cells and spread from cell-to-cell more effectively is not understood. Radiation is expected to induce cellular DNA repair mechanisms, and that may allow for more efficient synthesis of Ad DNA. Radiation may enhance the function of ADP. ADP probably functions by interacting with one or more cellular proteins, and radiation may affect this protein(s) such that ADP functions more efficiently.

It is believed that KD1, KD3, GZ1, or GZ3, or any other replication-competent Ad vector, when used in combination with radiation, will be more effective than vector alone or radiation alone in providing clinical benefit to patients with cancer. The vectors should allow more tumor destruction with a given amount of radiation. Stated another way, radiation should cause more tumor destruction with a given amount of vector. These vectors should also allow the radiation oncologist to use less radiation to achieve the same amount of tumor destruction. Less radiation would reduce the side effects of the radiation.

It is also believed that a cocktail of vectors when used in combination with radiation will be more effective than the cocktail alone or radiation alone. The cocktail could consist of ADP producing vectors plus one or more replication defective vectors expressing an anticancer therapeutic protein (see Example 5).

#### Example 9

This example illustrates a structure-function analysis of adenovirus death protein.

ADP is an 11.6 kDa N-linked O-linked integral membrane glycoprotein that localizes to the inner nuclear membrane (NM) (Scaria et al., Virology 191:743-753). As illustrated in Fig. 18, the Ad2-encoded ADP (SEQ ID NO:6) consists of 101 amino acids; aa 1-40 (SEQ ID NO:17) are luminal, aa 41-59 (SEQ ID NO:18) constitute the transmembrane signal-anchor

(SA) domain, aa 63-70 (SEQ ID NO:19) constitute a basic proline (BP) domain within the nucleoplasmic (NP) domain, which constitutes aa 61-101 (SEQ ID NO:20). To determine which domains in ADP are required to promote cell death, a number of deletion mutants of *rec700* were prepared which lacked various portions of the ADP gene and examined for the ability of ADP to localize to the NM and promote death. The *rec700* virus is an Ad5-Ad-Ad5 recombinant, which has been described elsewhere (Wold et al., *Virology* 148:168-180, 1986).

The structure of ADP in *rec700* and in each deletion mutant is schematically illustrated in Fig. 18. The ADP gene in each deletion mutant has been sequenced using PCR methods to insure that the mutations are correct. The structure and activity of ADP in the deletion mutants was tested by infecting A549 cells followed by immunoblot analysis of ADP mutant proteins as well as the ability to lyse cells. All deletion mutants expressed a stable ADP protein except *pm734.1* ( $\Delta 1-48$ , i.e. aa 1-48 are deleted). The *pm734.7* ( $N_{14}$ ) ADP, which has  $Asn_{14}$  mutated to Ser, is O-glycosylated but not N-glycosylated because  $Asn_{14}$  is the only N-glycosylation site (data not shown). The *d1735* ( $\Delta 4-11$ ) ADP is N-glycosylated but not O-glycosylated because the sites for O-glycosylation are deleted (data not shown). The *pm734.4* (M56) ADP, which has  $Met_{56}$  in the SA domain mutated to Ser, contains exclusively N-linked high-mannose oligosaccharides (data not shown); this occurs because the  $Met_{56}$  mutation precludes exit of ADP from the endoplasmic reticulum (ER). The *d1738* ADP, which lacks aa 46-60 in the signal-anchor domain, forms insoluble aggregates in the cytoplasm; therefore, aa 41-59 do in fact include the signal-anchor domain. The *pm734* ( $\Delta 1-40$ ) ADP, which initiates at  $Met_{41}$  at the N-terminus of the SA domain, comigrated with the lower group of bands generated by proteolytic processing (data not shown). This indicates that the proteolytic cleavage sites occur near  $Met_{41}$ . Consistent with this, the proteolytic products were not seen with *d1737* ( $\Delta 29-45$ ) (data not shown). Also, the size of the products decreased in all mutants with deletions within aa 41-101 (*d1715.1*, *d1715*, *d1714*, *d1716*) (data not shown).

The ability of these mutants to promote cell death was monitored by trypan blue exclusion, plaque development, and lactate dehydrogenase release assays (Tollefson et al., *J. Virol.* 70:2296-2306, 1996). The trypan blue results in Fig. 15A indicate that the death-promoting function of ADP was abolished by deletion of aa 1-40 (*pm734*), aa 11-26 (*d1736.1*), aa 18-22 (*d1735.1*), or aa 4-11 (*d1735*). Mutation of the N-glycosylation site at  $Asn_{14}$  (*pm734.7*) reduced the death-promoting activity to about 50% of *rec700* (WT). *d1737* ( $\Delta 29-45$ ) was efficient as *rec700* in promoting cell death; this indicates that the proteolytic processing products must not be required to promote cell death because they are not formed with *d1737*. The SA domain is essential for death because *d1738* ( $\Delta 46-60$ ) and *pm734.4*

(M56) were completely defective (Fig. 19). *dl715.1* was nearly completely defective, indicating that the BP domain is extremely important. Surprisingly, aa 71-94 (*dl714*), 76-89 (*dl715*), and 79-101 (*dl716*) could be deleted without affecting the death-promoting activity of ADP (Fig. 19). On the other hand, deletion of aa 81-88 (*dl717*) nearly completely  
 5 abolished the activity of ADP (Fig. 19); this is probably the result of aberrant sorting of ADP (see below). Similar results were obtained when the ability of these ADP mutants to promote cell death was examined with standard plaque development, LDH-release and MTT assays.

The effects of these mutations on the intracellular localization of ADP are extremely interesting. When examined by immunofluorescence (IF) at 33 h p.i. (data not shown), ADP  
 10 from *rec700* (WT) localized crisply to the NM; localization to the Golgi was also apparent. With *dl714* ( $\Delta$ 71-94) and *dl715* ( $\Delta$ 76-89), ADP localized to all membranes, i.e. the ER, Golgi, plasma membrane, and NM. This was even more apparent at 45 h p.i. (data not shown). Thus, aa 71-94 appear to include a signal that directs ADP specifically to the NM. ADP is very likely sorted from the *trans*-Golgi network (TGN) to the NM, so this putative signal in  
 15 ADP probably functions in this sorting pathway. ADP from *dl717* ( $\Delta$ 81-88) is intriguing: it localized to the NM and Golgi, but in many cells "dots" and circular structures were observed. Again, this was more apparent at 45 h p.i. when these structures were the prominent feature. *dl717*-infected cells have not begun to die at 45 h p.i., so these structures are not cellular remnants. The intriguing possibility is that these structures are membrane vesicles that have  
 20 pinched off from the TGN but are defective in targeting to and/or fusing with the NM.

With *dl738* ( $\Delta$ 46-60 in the SA domain), ADP aggregated in the cytoplasm. This again indicates that aa 46-60 include the SA sequence. With *pm734.4* (M56), ADP localized primarily to the NM. As discussed above, the *pm734.4* ADP has exclusively high-mannose N-linked oligosaccharides, indicating that it never leaves the ER. Perhaps the putative NM-  
 25 localization signal in the C-terminal region of the *pm734.4* ADP targets ADP to the NM by lateral diffusion from the ER (which is continuous with the outer and inner NM).

With *dl737* ( $\Delta$ 29-45), ADP localized to the NM. ADP from *pm734* ( $\Delta$ 1-40), *pm734.7* (N14) (N-linked glycosylation cannot occur), and *dl735* ( $\Delta$ 4-11; the O-glycosylation sites are deleted) localized much more prominently to the Golgi than the NM. ADP from *dl735.1*  
 30 ( $\Delta$ 18-22) and *dl736.1* ( $\Delta$ 11-26) also localized much more strongly to the Golgi than the NM. Thus, residues 1-26 and/or glycosylation appear to be required for efficient transport of ADP from the Golgi/TGN to the NM.

In summary, aa 41-59 include the SA domain, Met<sub>56</sub> in the SA domain is required for exit from the ER, aa 1-26 are required for efficient exit from the Golgi, and aa 76-94 are  
 35 required to target ADP specifically to the NM. With respect to promoting cell death, the

- essential regions are aa 1-26, the SA domain (ADP does not enter membranes), Met<sub>56</sub> in the SA domain, and the BP domain (aa 63-70). It is not clear whether the defective death-promoting phenotype of *pm734* ( $\Delta$ 1-40), *dl735* ( $\Delta$ 4-11), *dl735.1* ( $\Delta$ 18-22), *dl736.1* ( $\Delta$ 1-26), and *pm734.7* (N14) is due to lack of sequences (or oligosaccharides) that promote death or
- 5 much slower exit of ADP from the Golgi to the NM. *dl714* ( $\Delta$ 71-94) and *dl715* ( $\Delta$ 76-89) express a wild-type phenotype for promoting death even though they are defective in localizing specifically to the NM; this is probably because sufficient ADP still enters the NM to promote death. Even though the deletion in *dl717* ( $\Delta$ 81-88) lies within the deletions in *dl715* ( $\Delta$ 76-89) and *dl714* ( $\Delta$ 71-94), the *dl717* ADP is only about 15% as efficient as *rec700*
- 10 (WT), *dl715* and *dl714* in promoting death. This may be because the *dl717* ADP tends to remain in vesicles rather than localizing to the NM. Altogether, these data indicate that ADP must localize to the NM in order to promote cell death.

#### Example 10

- This example further characterizes the tissue specific Ad vectors described in Example 6. As
- 15 discussed therein, the Ad E4 promoter is deleted and replaced with the promoter for surfactant protein B (SPB) in these vectors (Figure 24).

#### Materials and Methods

- Cells, vectors and methods described in Example 6 were also used in this Example.
- In addition to the human cancer cell lines A549 (human lung carcinoma), Hep 3B (human
- 20 hepatocellular carcinoma), and H441 (papillary lung adenocarcinoma) used in Example 6, HEK 293 cells (obtained from Microbix (Toronto, ON)) and VK10-9 cells were used. VK10-9 cells are 293 cells that in addition to E1 contain and express E4 and pIX. These cells will be referred to as 293-E4 cells.

- Experiments employing phase contrast microscopy of Hep 3B and H441 cells were
- 25 performed as follows. Monolayers of Hep 3B or H441 cells were grown in 60 mm dishes with 5 ml of DMEM (10% FBS), and were mock-infected or infected with KD1 or KD1-SPB at a multiplicity of infection of 10 plaque forming units (PFU) per cell. Phase contrast photographs of monolayers were taken at 4 and 7 days postinfection (p.i.).

- Experiments employing western blots of H441 or Hep 3B cells were performed as
- 30 follows. H441 or Hep 3B cells (in 60 mm dishes) were infected with 10 PFU/cell of KD1 or KD1-SPB. At 24 h p.i., the cells were washed three times with PBS and harvested by scraping. The cells were lysed by RIPA buffer. The protein concentration was measured by the BIO-RAD DC Protein Assay Kit (BIO-RAD Laboratories, Hercules, CA) and 10  $\mu$ g of each sample were electrophoresed on 15% sodium dodecylsulfate polyacrylamide gels (SDS-
- 35 PAGE). The gels were electroblotted onto PVDF membranes (Immobilon, Millipore,



Bedford, MA). The membranes were blocked in TBST (50 mM Tris-Cl, pH 7.6, 150 mM NaCl, 0.2% Tween 20) containing 10% dry milk (Carnation) overnight at 4°C. After blocking, the membranes were incubated with a rabbit polyclonal antiserum against E4ORF3 (gift of Gary Ketner) or ADP (Tollefson et al., *J. Virol.* 66:3633-3642, 1992), or with M73, a  
5 monoclonal antibody against E1A (Harlow et al., *J. Virol.* 55:533-546, 1985). The secondary antibodies were goat anti-rabbit IgG-HRP or goat anti-mouse IgG-HRP. The blots were developed using the ECL protocol (Amersham Pharmacia, Arlington Heights, IL).

Experiments employing a lactate dehydrogenase release assay for cell lysis (Tollefson et al., *J. Virol.* 70:2296-2306) were performed as follows. H441 cells ( $7.7 \times 10^4$  cells per 35  
10 mm dish) and Hep 3B cells ( $9.0 \times 10^5$  cells per 35 mm dish) were infected at 20 PFU/cell in one ml serum-free DMEM. After an adsorption period of 1 h, 3 ml of DMEM (10% FBS) were added (final FBS concentration of 7.5%). Cells were incubated at 37°C with 6% CO<sub>2</sub>. At daily intervals, supernatants were collected, microfuged to remove floating cells, and cell-free supernatants were frozen at -70°C until assayed. Total lysis samples were prepared by  
15 addition of 10X lysis buffer included in the Cyto Tox 96 kit (Promega, Madison, WI). After all samples were collected, 20 µl samples were assayed in triplicate using the LDH assay kit Cyto Tox 96 and read on an EL340 Microplate reader (BioTec™ Instruments, Inc.) at 490 nm.

Experiments employing immunofluorescence evaluation of H441 and Hep 3B cells  
20 were performed as follows. H441 and Hep 3B cells were plated on Corning #1 coverslips in 35 mm dishes. H441 ( $1.5 \times 10^6$  cells/35 mm dish) and Hep 3B ( $9.0 \times 10^5$  cells/35 mm dish) were infected with 20 PFU/cell of the indicated viruses in 1 ml serum-free DMEM. After 1 h, 1 ml of DMEM/20% FBS was added (final concentration of 10% FBS). At the indicated  
25 times (48 h or 6 d p.i.), cells were fixed for 10 min in 3.7% paraformaldehyde in PBS, then permeabilized for 6 min in methanol (-20°C) and rehydrated in PBS. Coverslips were stained with rabbit antipeptide antiserum against the Ad E2A-coded DNA binding protein (DBP) (1:400 dilution; gift of Maurice Green) and mouse monoclonal antibody against fiber (1:400 dilution; gift of Jeff Engler) or were stained with rabbit antiserum to E4ORF3 (1:250 dilution; gift of Gary Ketner). Secondary antibodies (Cappel/ICN) were used at 1:50 dilution. All  
30 antibodies were diluted in PBS containing 1% BSA and 0.1% sodium azide. Photographs were taken on a Nikon epifluorescence microscope using a 100X Planapo lens and Tmax 400 film (Kodak). The film was developed in Diafine developer.

Analysis of viral DNA replication by Southern hybridization was performed as follows. H441 and Hep 3B cells were grown in 60 mm dishes in DMEM supplemented with  
35 10% FBS. Cells were infected at 70% confluence with 10 PFU/cell of KD1 or KD1-SPB.

Dishes were incubated in humidified 5% CO<sub>2</sub> atmosphere at 37°C. Total genomic DNAs were isolated at 5, 24, 48, 72, and 96 h p.i. Equal amounts of total genomic DNAs were digested with HindIII and resolved on a 1% agarose gel prior to transfer onto membranes. A random primer <sup>32</sup>P-labeled pBHG10 plasmid probe (Bett et al., *Proc. Natl. Acad. Sci. USA* 91:8802-8806, 1994) was used for hybridization, and the blots were autoradiographed. DNA fragments were quantitated on a Molecular Dynamics PhosphorImager.

Virus yields were determined as follows. Hep 3B cells or H441 cells grown as monolayers in 35 mm dishes were infected with 10 PFU/cell of KD1 or KD1-SPB. At days 0 to 4 (for H441) or days 0 to 9 (for Hep 3B) p.i., cells and culture medium were frozen at -70°C. Samples were frozen and thawed three times to release the virus from the cells, and total virus yields were determined by plaque assay on A549 monolayers.

The effect of KD1-SPB and KD1 on H441 and Hep 3B tumors was examined in a nude mouse model (Doronin et al., *J. Virol.* 74:6147-6155, 2000). Tumor cells (10<sup>7</sup> cells in 200 µl of DMEM, 50% Matrigel [Becton Dickinson Labware, Bedford, MA] for H441 cells, or 10<sup>7</sup> cells in 200 µl of DMEM plus 10% Matrigel for Hep 3B cells) were injected into flanks of 5-6 weeks old athymic nude mice and allowed to grow for three weeks to about 100 µl (H441) or 150 µl (Hep 3B) volumes. Pre-established tumors (n = 10) were injected with 50 µl of DMEM or 5 x 10<sup>7</sup> PFU of indicated viruses in DMEM. Injections of the viruses were repeated twice weekly for 3 weeks to the total dose of 3.0 x 10<sup>8</sup> PFU per tumor. Tumor size measurements were taken twice per week for H441 cells, or weekly for Hep 3B cells using a Sylvac digital caliper. Tumor volumes were calculated in according to the formula: length x width<sup>2</sup> / 2. Data are represented as means of increase in tumor size relative to the tumor size at the initial injection.

### Results

The properties of KD1-SPB in various cell types were compared to those of its "parent", KD1. Figure 25 shows the plaque development properties of these vectors on 293-E4, 293, and A549 cells. The data are plotted as the number of plaques seen on any day of the plaque assay as a percentage of the number of plaques seen at the end of the assay (i.e. when new plaques cease to appear) (Tollefson et al., *J. Virol.* 70:2296-2306, 1996). This assay is an indicator of the size of the plaques. KD1 formed plaques equally well on 293-E4 and 293 cells (Figure 25A). With KD1-SPB, plaques were observed about 3-4 days sooner on 293-E4 compared to 293 cells (Fig. 2A). On A549 cells, KD1 formed plaques 4-6 days sooner than KD1-SPB (Figure 25B).

The properties of KD1-SPB versus KD1 were characterized in detail in H441 cells, a human papillary lung adenocarcinoma cell line known to express the TTF1 transcription

factor and in which the SPB promoter is active (Yan et al., *J. Biol. Chem.* 270:24852-24857, 1995). Hep 3B cells, a human hepatocellular carcinoma in which the SPB promoter should not be active, were used as a negative control. H441 and Hep 3B monolayers were infected with 10 PFU/cell of KD1 or KD1-SPB and photographed at 4 and 7 days p.i. Mock-infected  
5 Hep 3B cells formed a relatively homogeneous monolayer, but H441 cells tended to form structures that resemble syncytia (Figure 26A, B). As expected, KD1 produced cytopathic effect (CPE) on both cell lines at 4 and 7 days p.i. (Figure 26A, B). Also as expected, KD1-SPB caused CPE on H441 cells but not on Hep 3B cells. Since CPE in Ad-infected cells is usually an indicator of virus growth, these results suggest that KD1-SPB grows in H441 but  
10 not in Hep 3B cells.

To examine viral DNA replication, H441 and Hep 3B cells were infected with 10 PFU/cell of KD1 or KD1-SPB, then the accumulation of viral DNA was determined by DNA blot. With H441 cells, KD1 and KD1-SPB DNAs were readily detected at similar levels at 48-96 h p.i. (Figure 27A). With Hep 3B cells, KD1 DNA levels were similar to those in  
15 H441 cells, but KD1-SPB DNA was barely detectable. This was confirmed by PhosphorImager analysis of the DNA bands (Figure 27B).

Growth of KD1-SPB and KD1 in H441 and Hep 3B cells was determined by a single step growth assay. Cells were infected with 10 PFU/cell of vector, then total vector yield was determined by plaque assay. Total yield of both vectors was similar in H441 cells, reaching a  
20 plateau after 2 days (Fig. 28A). KD1 yield plateaued in Hep 3B cells after 2-4 days p.i. (Figure 28B). However, KD1-SPB levels were about 5 logs lower in Hep 3B cells after 2-4 days, and even by 9 days they had not achieved the levels of KD1. We conclude that KD1-SPB grows with significant specificity on H441 versus Hep 3B cells. Further, KD1-SPB grows as well as KD1 on H441 cells, indicating that the E4 promoter deletion by itself does  
25 not significantly compromise the vector, and that the E4 promoter can be replaced by a tissue-specific promoter in a replication-competent vector.

To obtain further details on the replication of KD1-SPB vs KD1 in H441 and Hep 3B cells, the expression of representative Ad proteins by KD1-SPB and KD1 was examined. H441 or Hep 3B cells were mock-infected or infected with 10 PFU/ml of KD1 or KD1-SPB,  
30 then at 24 h p.i. the proteins were extracted and the E1A, E4ORF3, and ADP proteins were examined by immunoblot. E4ORF3 is one of the six proteins coded by the E4 transcription unit (Leppard, *J. Gen. Virol.* 78:2131-2138, 1997). As anticipated, KD1-SPB expressed E4ORF3 well in H441 cells, but only at trace levels in Hep 3B cells (Figure 29). KD1-SPB expressed the E1A proteins in Hep 3B cells. Synthesis of E1A proteins by KD1-SPB in Hep  
35 3B cells is expected because E1A expression does not require E4 proteins; it also indicates

that the block to infection with KD1-SPB is downstream of E1A. KD1 expressed E1A in both cell lines, but the amount was less than obtained with KD1-SPB in Hep 3B cells (Figure 29). The increased E1A levels seen with KD1-SPB may reflect its poor ability to enter the late phase of infection (see Discussion). KD1-SPB expressed ADP as well as KD1 in H441  
5 cells, but it did not make detectable ADP in Hep 3B cells. ADP is primarily a late protein, so this result is consistent with the relative lack of E4 protein expression, DNA replication, and growth of KD1-SPB in Hep 3B cells.

To gain insights into replication events that occur in individual cells, expression of E4ORF3, the E2A-DBP, and the fiber late protein was examined by immunofluorescence.  
10 H441 or Hep 3B cells were infected with 20 PFU/cell. At 48 h or 6 days p.i., cells were fixed and immunostained. E4ORF3 was detected in the nuclei of H441 cells at 48 h p.i. with KD1, KD1-SPB, or dl309 (Figure 30A). (dl309 is an Ad5 mutant that has wild-type E1A, expresses Ad5 levels of ADP, and lacks the E3-RID and E3-14.7K genes). E4ORF3 could not be detected in the vast majority of Hep 3B cells infected with KD1-SPB (Figure 30A), even at 6  
15 days p.i. (Figure 30B). Thus, KD1-SPB expresses E4ORF3 well in H441 but not in Hep 3B cells.

Figure 31A shows double label immunofluorescence of DBP and fiber in the same Hep 3B cells at 48 h p.i. with KD1 or KD1-SPB. With KD1, there was a strong speckled staining pattern in the nucleus that is typical for DBP at 48 h p.i. (Figure 31A, top left panel).  
20 There was strong staining of fiber throughout these same cells (Figure 31A, top right panel). Staining of the cytoplasm and nucleus is expected because fiber is synthesized in the cytoplasm and then transported to the nucleus where virions assemble. With KD1-SPB at 48 h p.i., about 25% of the cells showed the speckled staining for DBP, and only one cell (7% of total) with the advanced speckled pattern was also stained for fiber (Figure 31A, bottom two  
25 panels). Even at 6 days p.i., only about 30% of cells showed staining for DBP, and about 20% for fiber (Figure 31B). Thus, markedly fewer Hep 3B cells infected with KD1-SPB expressed DBP and especially fiber as compared to KD1. These results indicate that KD1-SPB replicates as well as KD1 in H441 cells, no doubt because the SPB promoter is active in H441 cells (Yan et al., *J. Biol. Chem.* 270:24852-24857, 1995). KD1-SPB barely replicates  
30 in Hep 3B cells, presumably because the SPB promoter is minimally active in these cells.

At the culmination of replication, Ad-infected cells are lysed and the virus spreads to other cells; this process is mediated in large part by ADP (Tollefson et al., *Virology* 220:152-162, 1996; Tollefson et al., *J. Virol.* 70:2296-2306, 1996). To examine vector-induced cell lysis, H441 and Hep 3B cells were mock-infected or infected with 20 PFU/cell of KD1, KD1-  
35 SPB, or dl309, and cell lysis was determined by release of lactate dehydrogenase (Tollefson et

al., *J. Virol.* 70:2296-2306, 1996). All vectors lysed H441 cells beginning at 2-3 days p.i. (Figure 32A). KD1 and d1309 also lysed Hep 3B cells in the same time period; however, KD1-SPB caused only minimal cell lysis (Figure 9B). Thus, these data, along with the cell spread data in Example 6 and Figure 13, demonstrate that KD1-SPB lyses cells and spreads efficiently from cell-to-cell in H441 but not Hep 3B cells.

An experiment was conducted to determine whether KD1-SPB or KD1 would suppress H441 tumors in nude mice. H441 cells were injected into each hind flank. When tumors had grown to about 100  $\mu$ l (H441) or 150  $\mu$ l (Hep 3B), they were injected twice weekly for 3 weeks with DMEM (mock) or  $5 \times 10^7$  PFU of test virus in 50  $\mu$ l of DMEM ( $3.0 \times 10^8$  total PFU). Ten tumors (5 mice) were used for each virus. Growth of H441 tumors was suppressed similarly by KD1-SPB and KD1 (Figure 33A). KD1 suppressed growth of Hep 3B tumors, whereas KD1-SPB caused only minimal suppression (Figure 33B). These results show that KD1-SPB is as effective as KD1 in suppressing tumors when the SPB promoter is active. Further, the cell type specificity observed with KD1-SPB in vitro is maintained in vivo.

#### Discussion

Tumor specificity is one of the biggest challenges facing cancer gene therapy, i.e. having the therapeutic gene be expressed specifically in cancer cells. Specificity is very important for RC viruses. Two main strategies have been described that in theory confer specificity: transductional targeting and transcriptional targeting. Directing specificity of vectors toward specific cell surface receptors on the target cells has been attempted through various methods. Although this approach is theoretically attractive it might encounter multiple obstacles such as the lack of incorporation of the engineered protein into the virion (Scaria et al., *Virology* 191:743-753, 1992) or lack of infectivity through the targeted receptor (Cosset et al., *J. Virol.* 69:6314-6322, 1995). Transcriptional targeting utilizes tumor and tissue specific promoters. In replication-defective vectors these regulatory sequences confine the expression of cytotoxic genes to specific tissues. In replication-competent vectors, as an added layer of regulation, vector replication per se can be placed under the control of tumor or tissue specific promoter/enhancer sequences. In replication-competent Ad, insertion of the tissue or tumor specific promoter/enhancer into the E1A promoter/enhancer region has been used exclusively (Hallenbeck et al., *Hum. Gene Ther.* 10:1721-1733, 1999; Rodriguez et al., *Cancer Res.* 57:2559-2563, 1997; Yu et al., *Cancer Res.* 59, 4200-4203, 1999; Yu et al., *Cancer Res.* 59:1498-1504, 1999). The rationale behind these vectors is that expression of E1A and therefore the whole Ad transcription program will depend on these tissue or tumor specific promoters. However, as a generic approach, there may be difficulties. The E1A

enhancer/promoter is very complex. The enhancer controls not only the E1A promoter but also distant promoters such as the E4 promoter (Shenk, T. pp. 2111-2148 *In* B.N. Fields, D.M. Knipe, and P.M. Howley (eds.), Fields Virology, Lippincott-Raven, Philadelphia, 1996). In addition, it has been shown that the E1A enhancer in the inverted terminal repeat region changes tissue specificity of cellular promoters (Shi et al., *Hum. Gene Ther.* 8:403-410, 1997). Also, the E1A enhancer/promoter is partially embedded within the signals required to package the Ad genome into virions, and it may be problematic to remove all the E1A enhancer elements without impairing virus production. Accordingly, we chose to replace the E4 promoter with a tissue specific promoter. E4 genes are essential for Ad replication, and therefore we expected that the replication of the recombinant virus would be dependent on the tissue specific regulatory elements.

To construct KD1-SPB, the ca. 300 bp of the E4 promoter was deleted and the B-500 version (ca. 500 bp) of SPB promoter was inserted (Yan et al., *supra*) (Figure 24 C, D). We selected the SPB promoter because of its strict tissue specificity: it is exclusively active in type II alveolar cells and bronchial epithelial cells of the lung (Bohinski et al., 1994, *Mol. Cell. Biol.* 14:5671-5681, 1994). Since the parental virus KD1 contains and expresses two E1A mutations that restrict virus replication to tumor cells (Doronin et al., *supra*), we anticipated that the virus would selectively replicate in cells derived from lung tumors. Thus, H441 cells, a papillary lung carcinoma cell line, were used to characterize the replication, gene expression, and functional profile of KD1-SPB.

KD1-SPB formed plaques 3-4 days sooner on 293-E4 cells that express E4 proteins than on 293 cells, whereas KD1 formed plaques with the same kinetics on both cell lines. These data show that the E4 promoter is active in 293 cells, and that the SPB promoter displays very low activity in 293 cells. It is not clear why KD1-SPB forms plaques on 293 cells; these cells are derived from human embryonic kidney and at least one of the transcription factors regulating the SPB promoter (Bohinski et al., *supra*), hepatocyte nuclear factor 3, is expressed in embryonic kidney. It is also possible that TTF1, the master regulatory factor of SPB expression, is minimally active in 293 cells.

KD1 grew to equally high titers in H441 and Hep 3B cells (Figure 28A, B). In contrast, KD1-SPB replicated as efficiently as KD1 in H441 cells, in which the SPB promoter is active (Yan et al., *supra*) (Figure 28A), but replicated poorly in Hep 3B cells, most likely because the SPB promoter is inactive (Figure 28B). This selectivity has been confirmed by measuring viral DNA production in the two cell lines. KD1-SPB DNA replication was similar both kinetically and quantitatively to KD1 DNA replication in H441, however in Hep

3B cells, KD1-SPB DNA was almost undetectable (Figure 27A, B). The cytopathic effect, a surrogate marker of Ad replication, showed a similar specificity (Figure 26).

To further confirm our predictions on the molecular basis of the observed tissue specificity we monitored viral protein expression. When cells were infected with KD1-SPB all the viral proteins early or late, except for E1A, were expressed in a tissue-specific fashion (high expression in H441, low to undetectable expression in Hep 3B) (Figures 29-31). We found a good correlation between the levels of E4 promoter activity (E4ORF3 expression) and the expression of E2A-DBP, ADP, and fiber proteins. Thus, the SPB promoter retains its tissue specificity in the Ad genome and it seems to be the limiting factor of Ad gene expression in the cell lines tested. As expected, expression of E1A is not tissue-specific. Thus, the regulatory step of tissue-specific Ad DNA replication is downstream of E1A. In Hep 3B cells, KD1-SPB expressed E1A at a higher level than did KD1 (Figure 29), strongly suggesting that KD1-SPB replication in most of the Hep3B cells remains at the early stage.

The cytolytic effect of KD1-SPB also showed a tissue-specific profile (Figure 32; Figure 13 of Example 6), i.e., preferential lysis of H441 cells over Hep 3B cells, a pattern similar to the specificity observed at the level of DNA replication (Figure 27) and viral protein synthesis (Figures 29-31). This cell type specificity was also observed when these cells were growing as tumors in nude mice. Growth of H441 tumors was suppressed by KD1-SPB and KD1 at similar efficacy (Figure 33A). In contrast, KD1-SPB unlike KD1 had only minimal effect on the growth of Hep 3B tumors (Figure 33B).

In summary, substitution of the E4 promoter with a tissue specific promoter allows highly tissue specific replication of Ad vectors and in the target tissue it is as efficient as the replication of the parental virus. KD1-SPB lacks all E3 genes except ADP. E3 gp19K, RID and 14.7K have been shown to protect Ad-infected cells from attack by cytotoxic lymphocytes and apoptosis-inducing cytokines such as tumor necrosis factor and Fas ligand (Wold et al., pp. 200-232 *In A.J. Cann (ed.), DNA Virus Replication: Frontiers in Molecular Biology*, Oxford University Press, Oxford, 2000; Wold et al., *Curr. Opin. Immunol.* 11:380-386, 1999).

The therapeutic index (virus produced in H441 cells compared to Hep 3B cells) of KD1-SPB is  $10^4$ - $10^5$  for the first 4-5 days (Figure 28). These data compare to data reported by Calydon ( $10^4$ - $10^5$ ) for their prostate specific viruses (Rodriguez et al., *supra*; Yu et al., *Cancer Res.* 59, 4200-4203, 1999; Yu et al., *Cancer Res.* 59:1498-1504, 1999). We suggest that KD1-SPB has some added advantage over vectors reported by other laboratories because it encodes a mutant form of E1A that restricts replication to cancer cells (Doronin et al., *supra*).

Although the lung ranks as the second highest cancer site for both men and women in the U.S. Reis et al., *Cancer Res.* 88:2398-2424, 2000), lung cancer has not been a major target for cancer vector gene therapy since intratumoral injection of virus is generally not feasible in the lungs. However, there has been a recent report of intratumor injection of a replication-  
5 defective Ad vector into a lung tumor, and such an approach could be attempted with KD1-SPB. It may also be feasible to administer KD1-SPB systemically in the lung.

In view of the above, it will be seen that the several advantages of the invention are achieved and other advantageous results attained.

As various changes could be made in the above methods and compositions  
10 without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification, including patents and patent  
applications, are hereby incorporated by reference. The discussion of references herein is  
15 intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.



What is Claimed Is:

1. A recombinant vector which is replication-competent in a neoplastic cell and which overexpresses an adenovirus death protein.
2. The recombinant vector of claim 1 wherein the adenovirus death protein comprises amino acids 1-26, 41-59, and 63-70 of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 or a conservatively substituted variant thereof or wherein the adenovirus death protein comprises SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8.
3. The recombinant vector of claim 2 which comprises a recombinant virus.
4. The recombinant vector of claim 3, wherein the recombinant virus is an adenovirus lacking expression of at least one E3 protein selected from the group consisting of: gp19K; RID $\alpha$ ; RID $\beta$  and 14.7K.
5. The recombinant vector of claim 4 which comprises SEQ ID NO:3 or SEQ ID NO:4.
6. The recombinant vector of claim 3 which is replication-restricted to neoplastic cells.
7. The recombinant vector of claim 6 which comprises SEQ ID NO:1 or SEQ ID NO:2.
8. The recombinant vector of claim 3, wherein the recombinant adenovirus comprises a tissue specific promoter, a tumor specific promoter, or an inducible promoter substituted for the E4 promoter.
9. The recombinant vector of claim 8, wherein the tissue-specific promoter is a surfactant protein B promoter.
10. The recombinant vector of claim 6 which comprises SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.
11. The recombinant vector of claim 1, wherein the vector further comprises a gene encoding an anti-cancer product.
12. The recombinant vector of claim 11, wherein the gene encoding an anti-cancer product is in the E3 region of the vector.
13. A method for promoting death of a neoplastic cell comprising contacting the neoplastic cell with at least one vector which is replication competent in the neoplastic cell and which overexpresses an adenovirus death protein.
14. The method of claim 13 wherein the adenovirus death protein comprises amino acids 1-26, 41-59, and 63-70 of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ

ID NO:8 or a conservatively substituted variant thereof or wherein the adenovirus death protein comprises SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8.

15. The method of claim 14, wherein the vector comprises a recombinant adenovirus lacking expression of at least one E3 protein selected from the group consisting of: gp19K; RID $\alpha$ ; RID $\beta$  and 14.7K.

16. The method of claim 15, wherein the neoplastic cell comprises a tumor in a patient and the contacting step comprises administering the recombinant adenovirus to the tumor.

17. The method of claim 16, further comprising the step of passively immunizing the patient against the recombinant adenovirus.

18. The method of claim 17, wherein the recombinant adenovirus comprises SEQ ID NO:3 or SEQ ID NO:4.

19. The method of claim 15, wherein the vector is replication-restricted to neoplastic cells.

20. The method of claim 19, wherein the vector is a recombinant adenovirus comprising SEQ ID NO:1 or SEQ ID NO:2.

21. The method of claim 15, wherein the recombinant adenovirus comprises a tissue specific promoter or an inducible promoter substituted for the E4 promoter.

22. The method of claim 21, wherein the tissue specific promoter is a surfactant protein B promoter.

23. The method of claim 22, wherein the recombinant adenovirus comprises SEQ ID NO:14, SEQ ID NO:15 or SEQ ID NO:16.

24. The method of claim 16, further comprising treating the tumor with radiation.

25. The method of claim 24, comprising administering more than one recombinant adenovirus to the tumor and treating the tumor with radiation.

26. The method of claim 16, further comprising treating the tumor with chemotherapy.

27. The method of claim 26, comprising administering more than one recombinant adenovirus to the tumor and treating the tumor with chemotherapy.

28. The method of claim 16, further comprising administering to the tumor one or more replication-defective adenovirus which expresses an anti-cancer gene product, wherein the recombinant adenovirus complements spread of the replication-defective adenovirus in the tumor.

29. A composition comprising:

a first recombinant virus which is replication competent in a neoplastic cell and overexpresses an adenovirus death protein; and

a second recombinant virus which is replication defective and which expresses an anti-cancer gene product,

wherein the first recombinant virus complements replication of the second recombinant virus.

30. The composition of claim 29 wherein the first recombinant virus comprises a recombinant adenovirus lacking expression of at least one E3 protein selected from the group consisting of: gp19K; RID $\alpha$ ; RID $\beta$  and 14.7K.

31. The composition of claim 30 wherein the recombinant adenovirus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:14; SEQ ID NO:15; SEQ ID NO:16; SEQ ID NO:3; or SEQ ID NO:4.

32. A composition comprising

a first recombinant virus which is replication-defective in a neoplastic cell and which overexpresses an adenovirus death protein, and

a second recombinant virus which is replication-competent in a neoplastic cell.

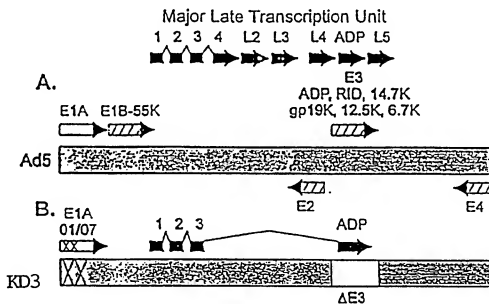


FIGURE 1

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# ADP Is Expressed Earlier in Infection By KD1, KD3, GZ1, and GZ3

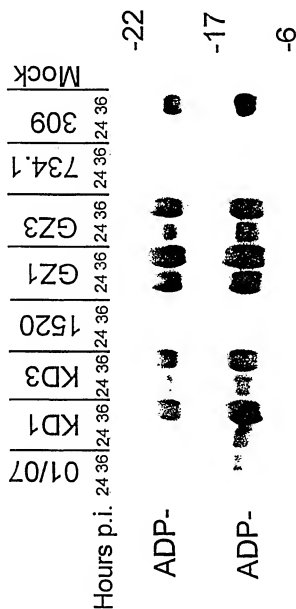


FIGURE 2

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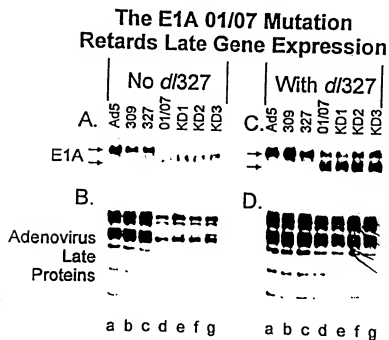


FIGURE 3

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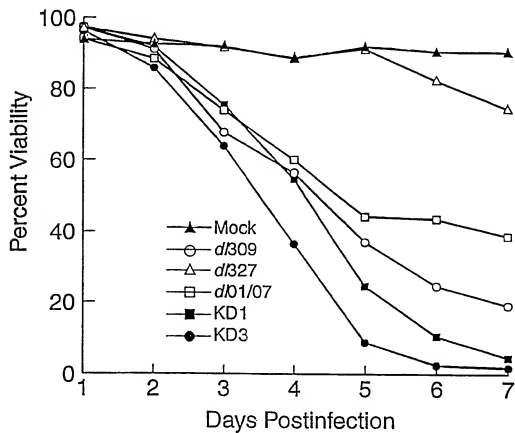


FIGURE 4

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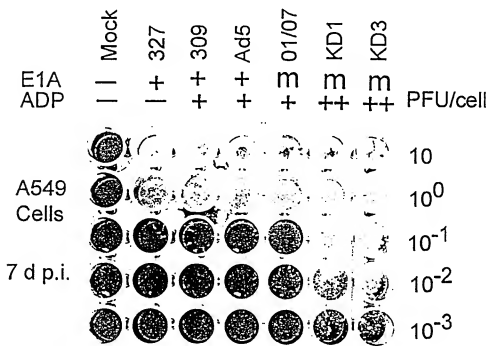


FIGURE 5

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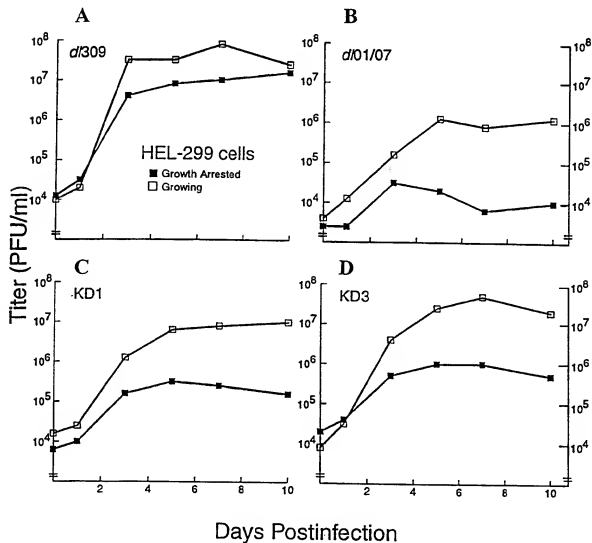


FIGURE 6

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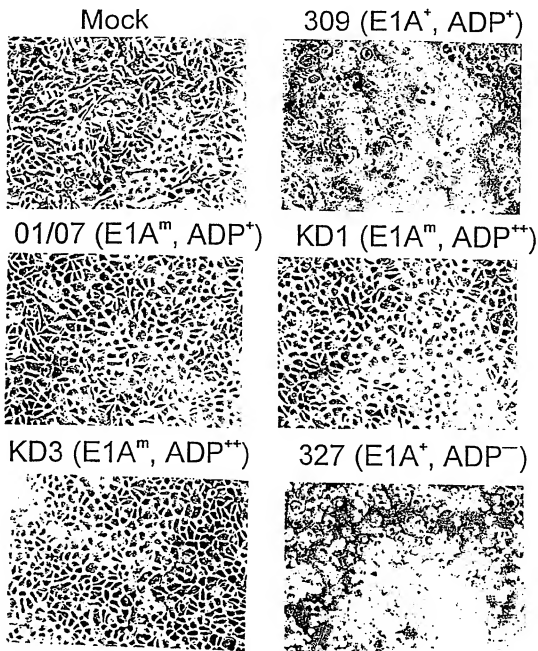


FIGURE 7

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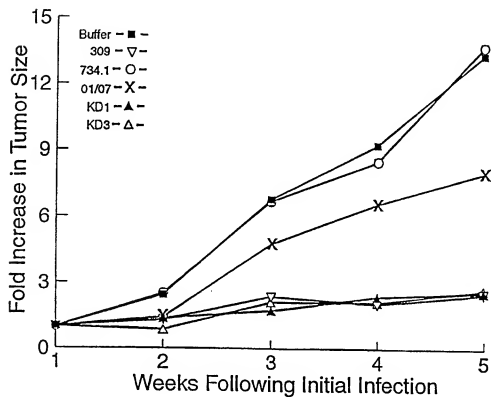


FIGURE 8A

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One Injection of KD3 or GZ3 Inhibits  
Growth of A549 tumors  
( $5 \times 10^8$  PFU injected on day 0)

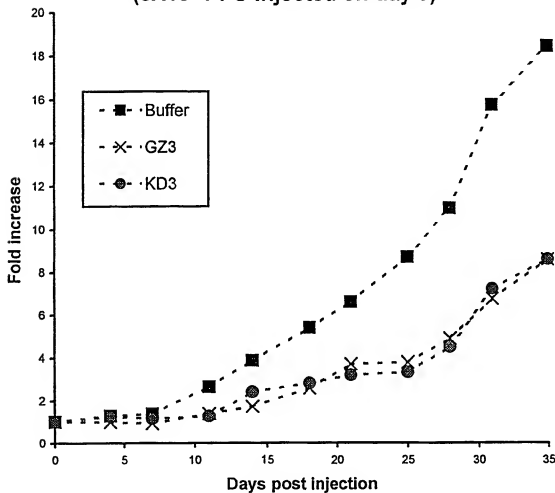


FIGURE 8B

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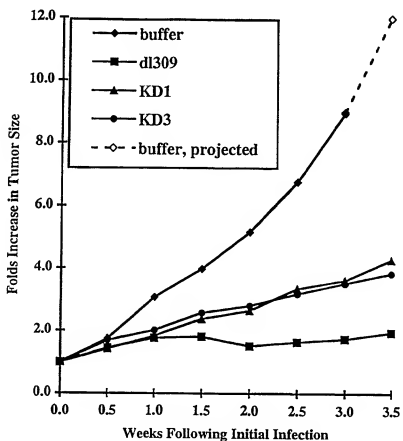


FIGURE 9

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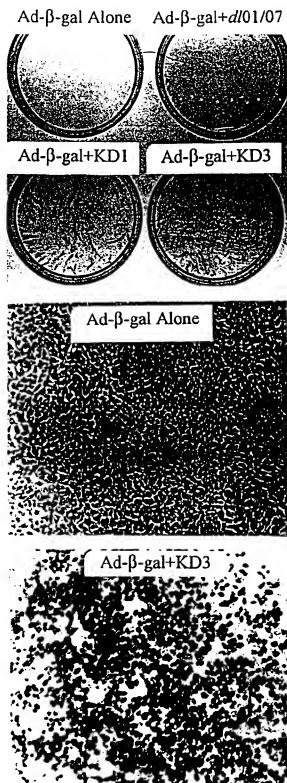


FIGURE 10

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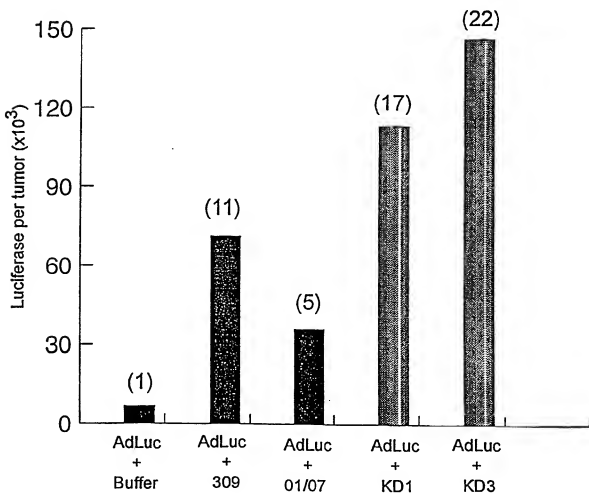


FIGURE 11

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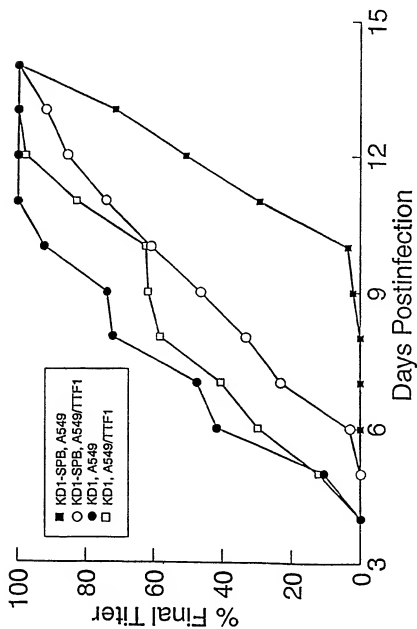
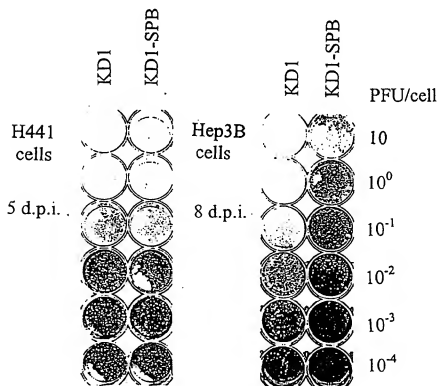


FIGURE 12

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**KD1-SPB With the SPB Promoter in Place of the E4  
Promoter Grows on H44a Lung Cancer Cells with the  
TTF1 Transcription Factor**



**FIGURE 13**

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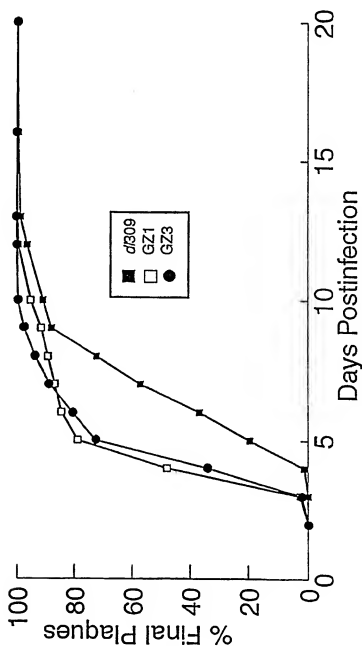


FIGURE 14

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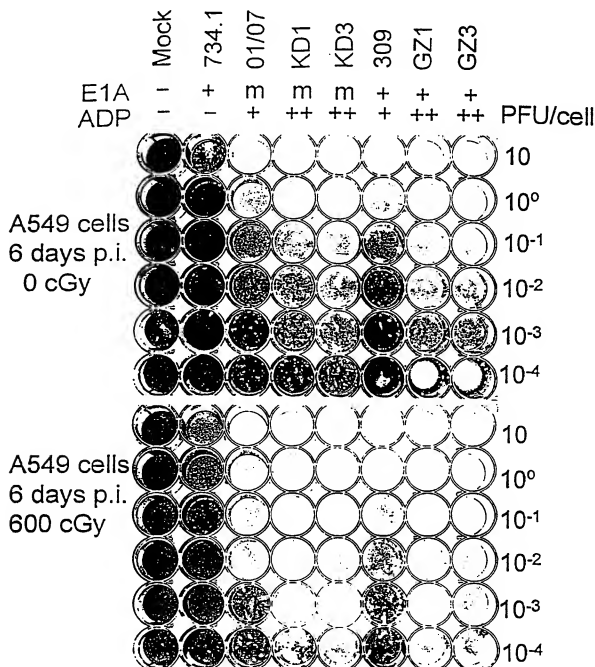


FIGURE 15

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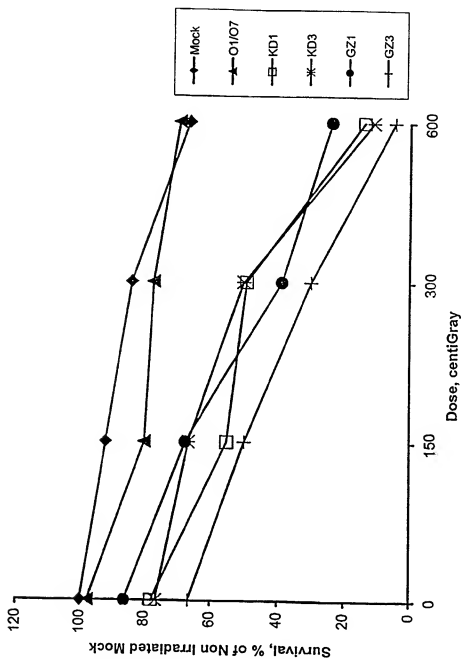


FIGURE 16

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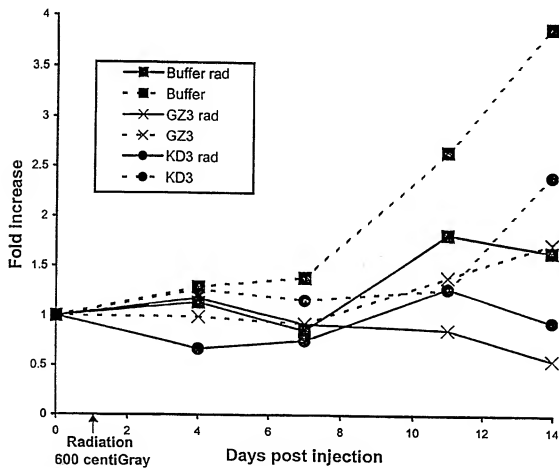


FIGURE 17

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## Ad2 Adenovirus Death Protein

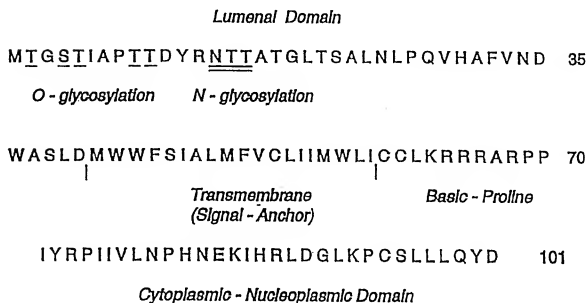


FIGURE 18A

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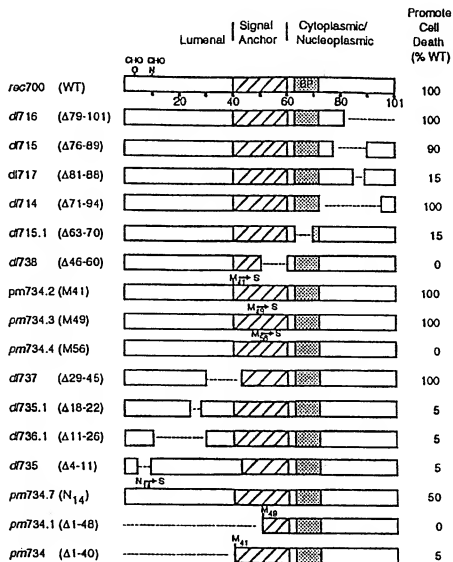


FIGURE 18B

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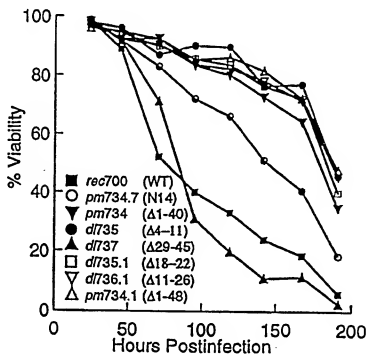


FIGURE 19A

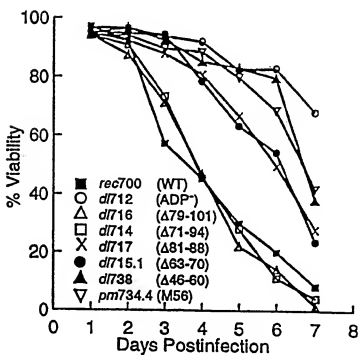


FIGURE 19B

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## Seq ID No.

	10	20	30	40	50
5 Ad1	-----MVD	T VNSYNTATGL	TSALNLPQVS	TFVNNWANLG	MWWSFIALMF
6 Ad2	MTGSTIAPT	DYRNTTATGL	TSALNLPQVH	AFVNDWASLD	MWWSFIALMF
7 Ad5	-----MTN	TTNAAATGL	TSTTNTQVS	AFVNNWDNLG	MWWSFIALMF
8 Ad6	-----MVD	T VNSYNTATGL	KSALNLPQVH	AFVNDWASLG	MWWSFIALMF
9 dI716	MTGSTIAPT	DYRNTTATGL	TSALNLPQVH	AFVNDWASLD	MWWSFIALMF
10 dI715	MTGSTIAPTT	DYRNTTATGL	TSALNLPQVH	AFVNDWASLD	MWWSFIALMF
11 dI714	MTGSTIAPTT	DYRNTTATGL	TSALNLPQVH	AFVNDWASLD	MWWSFIALMF
12 dI737	MTGSTIAPTT	DYRNTTATGL	TSALNLPQ--	-----	-----IALMF

	60	70	80	90	100
5 Ad1	VCLIIIMWLS	CLKRRRARPP	IYKPIIVLNP	NNDGIHRLDG	LNTCSFSPAV -
6 Ad2	VCLIIIMWLIC	CLKRRRARPP	IYRPIIVLNP	HNEKIHRLDG	LKPCSLLQY D
7 Ad5	VCLIIIMWLIC	CLKRRRARPP	IYSPPIVLHP	NNDGIHRLDG	LKGMFFSLTV -
8 Ad6	VCLIIIMWLIC	CLKRRRARPP	IYRPIIVLNP	HNEKIHRLDG	LKPCSLLQY D
9 dI716	VCLIIIMWLIC	CLKRRRARPP	IYRPIIVL--	-----	-----
10 dI715	VCLIIIMWLIC	CLKRRRARPP	IYRPI-----	-----G	LKPCSLLQY D
11 dI714	VCLIIIMWLIC	CLKRRRARPP	-----	-----	-----SLLQY D
12 dI737	VCLIIIMWLIC	CLKRRRARPP	IYRPIIVLNP	HNEKIHRLDG	LKPCSLLQY D

## Seq. ID No.

17	aa 1-40 of Ad2 ADP	MTGSTIAPTT DYRNTTATGL TSALNLPQVH AFVNDWASLD
18	aa 41-59 of Ad2 ADP	MWWSFIALMF VCLIIIMWLI
19	aa 63-70 of Ad2 ADP	KRRRARPP
20	aa 60-101 of Ad2 ADP	C CLKRRRARPP IYRPIIVLNP HNEKIHRLDG LKPCSLLQY D

FIGURE 20

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LOCUS ad5 comple 35935 bp DNA SYN 06-FEB-1999  
 DEFINITION ad5 complete genome  
 ACCESSION ad5 comple  
 KEYWORDS  
 SOURCE Unknown.  
 ORGANISM Unknown.  
 Unclassified.  
 REFERENCE 1 (bases 1 to 35935)  
 AUTHORS Self  
 JOURNAL Unpublished.  
 BASE COUNT 8367 a 10073 c 9761 g 7734 t  
 ORIGIN  
 1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG GGGGTGGAGT  
 61 TTGTGACGTC GCGCGGGGCG TGGGAACGGG GCGGGTGACG TAGTAGTGTC GCGGAAGTGT  
 121 GATGTTGCAA GTGTGCGGGA ACACATGTAA GCGACGGATG TGGCAAAAGT GACGTTTTTG  
 181 GTGTGCGCCG GTGTACACAG GAAGTGACAA TTTTCGCGCG GTTTTAGCGG GATGTTGTAG  
 241 TAAATTTGGG CGTAACCGAG TAAGATTGCG CCATTTTCGC GGGAAAACTG AATAAGAGGA  
 301 AGTGAATCTT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTGTGTCTA TTTTACCGGG  
 361 GACITTTGACC GTTTACGTTG AGACTCGCCC AGGTGTTTTT CTCAGGTGTT TCCCGGTTTC  
 421 CGGGTCAAGT TTGGCGTTTT ATTATTATAG TCAGCTGACG TGTAGTGTAT TTATACCGGG  
 481 TGAGTCTCTC AAGAGGCCAC TCTTGAGTGC CAGCGAGTAG AGTTTCTTCC TCCGAGCGCG  
 541 TCCGACACCG GACTGAAAA TGAGACATAT TATCTGCCAC GGAGGTGTTA TTACCAGAAG  
 601 AATGGCGGCC AGTCTTTTGG ACCAGCTGAT CSAAGAGTA CTGGCTGATA ATCTTCCACC  
 661 TCTTAGCCAT TTTGAACCC CTAACCTTCA CGAAGTGTAT GATTTAGAGC TGACGGCGCC  
 721 CGAAGATCCC AACGAGGAGG CGGTTTCGCA GATTTTTCCC GACTCTGTAA TGTTCGCGGT  
 781 GCAGGAAGGG ATTGACTTAC TCACTTTTCC GCGCGCGCCC GGTTCTCGGG AGCCGCTCA  
 841 CTTTTCGCGG CAGCCCGAGC AGCCCGAGCA GAGAGCGCTG GTCCCGGTTT CTATGCCAAA  
 901 CTTGTGACCG GAGGTGATCG ATCTTACCTG CCACGAGGCT GGCTTTCCAC CCAAGTACGA  
 961 CGAGGATGAA GAGGCGTAGG AGTTTGTGTT AGATTATGTG GAGCACCCCG GGACCGGTTG  
 1021 CAGGTCTTGT CATTTACACC GGAGGAATAC GGGGGACCCA GATATTATGT GTTCGCTTTG  
 1081 CTATATGAGG ACCTGTGGCA TGTTTGTCTA CAGTAAAGTA AAATTATGGG GACTGGGTGA  
 1141 TAGAGTGGTG GGTTTGGTGT GGTAATTTT TTTTAAATTT TTACAGTTTT GTGGTTTAAA  
 1201 GAATTTTGTA TTGTGATTTT TTTAAAGGTT CCGTGTCTG AACCTGAGCC TGAGCCCGAG  
 1261 CCAGAACCGG AGCCTGCAAG ACCTACCCGC CGTCCCTAAA TGCGCGCTGC TATCCTGAGA  
 1321 CGCCCGACAT CACCTGTGTC TAGAGAATGC AATAGTAGTA CGGATAGCTG TGACTCCGTT  
 1381 CTTCTAACA CACCTCTCTG GATACACCGG GTGGTCCCGC TGTGCCCAT TAACACAGTT  
 1441 GCGGTGAGAG TTGGTGGGCG TCGCCAGGCT TGGAATGTA TCGAGGACTT TGCTACCGAG  
 1501 CCGGCGCAAC CTTTGGACTT GAGCTGTAAA CGCCCGAGGC CATAGGTTGT AAACCTGTGA  
 1561 TTGCGTGTGT GGTAAACGCC TTTGTTTGCT GAATGAGTTG ATGATAGTTT AATAAGGGT  
 1621 GAGATAATGT TTAACCTGCA TGCGCTGTTA AATGGGCGCG GCGCTTAAAGG GTATATAATG  
 1681 GCGCGTGGGC TAATCTTGTT TACATCTGAC CTCATGGAGG CTTGGGAGTG TTTGGAAGAT  
 1741 TTTTCTGCTG TGCCTAACCT GCTGGAACAG AGCTCTAACA GTACCTCTTG GTTTTGGAGG  
 1801 TTTCTGTGGG GCTCATCCCA GGCAAGTATA GTCTGCAGAA TTAAGGAGGA TTACAGTGTG  
 1861 GAATTTGAAG AGCTTTTGA AATCTGTGTT GAGCTGTGTT ATCTTTGATA TCTGGTTCAC  
 1921 CAGGCGCTTT TCCAAGAGAA GGTCTACAG ACTTTGGATT TTTCCACACC GGGGCGCGGT  
 1981 CGCGCTGCTT TTGCTTTTTT GAGTTTATA AAGGATAAAT GAGAGCGGAT AACCCATCTG  
 2041 AGCGGGGGGT ACCTGCTGGA TTTTCTGGCC ATGCACTGTG GGAGAGCGGT TGTGAGACAC  
 2101 AAGATCTGCC TGCTACTGTT GTCTTCGCTG CGCCCGGCGA TAATACCGAT TAAGAGCGAG  
 2161 CAGCAGCAGC AGGAGGAAGC CAGGCGGCGG CGGCAGGAGC AGAGCCCATG GAACCCGAGA  
 2221 GCGCGCTGGG ACCCTCGGGA ATGAATGTTG TACAGGTGGC TGAACCTATG CCAGACCTGA  
 2281 GACGCATTTT GACAATTACA GAGGATGGGC AGGGGCTAAA GGGGGTAAAG AGGAGCGGGG  
 2341 GGGCTGTGGA GCGTACAGAG GAGGCTAGGA ATCTAGCTTT TAGCTTAATG ACCAGAGCCT  
 2401 GTCTGAGTGT TATTACTTTT CAACAGATCA AGGATAATTG CGCTAATGAG CTTGATCTGC  
 2461 TTGGCGCAGG GTATTCCATA GAGCAGCTGA CCACTTACTG GCTGCAAGAG GGGGATGATT  
 2521 TTGAGGAGGC TATTAGGGTA TATGCAAAGG TTGCAATTCG CCGAGATTGC AAGTACAAGA  
 2581 TCAGCAAACT TGTAAATATC AGGAATTGTT GCTACATTTT TGGGAACCGG GCGGAGGTGT  
 2641 AGATAGATAC GGAGGATAGG GTGGCTTTTA GATGTAGCAT GATAAATATG TGGCCGGGGG

ad5

 FIGURE 21  
 (SHEET 1)

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2701 TGCTTGGCAT GGACGGGGTG GTTATTATGA ATGTAAGGTT TACTGGCCCC AATTTTAGCG
2761 GTACGGTTTT CTTGGCCAAAT ACCAACCTTA TCCTACACGG TGTAAAGCTTC TATGGGTTTA
2821 ACAATPACCTG TTGGGAAGCC TGGACCGATG TAAGGGTTCC GGGCTGTGCC TTTTACTGCT
2881 CTGTGAAGGG GTTGGTGTGT CQCCCAAAAG GCAGGGCTTC AATTAAAGAA TGCTCTTTTG
2941 AAAGGTGTAC CTTGGGTATC CTGTCTGAGG GTAACTCCAG GATTAAGCAT AACATGGTAT
3001 CCGACTGTGG TTGCTTCATG CTAGTGAATA GCGTGGCTGT GATTAAAGCAT AACATGGTAT
3061 GTGGCAACTG CGAGGACAGG GCCTCTCAGA TGCTGACCTG CTCGGACGGC AACTGTACAC
3121 TGCTGAAGAC CATTACGTA GCCAGCCACT CTCGCAAGCG CTGGCCAGTG TTTTGGACATA
3181 ACATACTGAC CCGCTGTTC TTGCATTGGG GTAAACAGAG GGGGGTGTTC CTACCTTACC
3241 AATGCAATTG GAGTCACACT AAGATATTGC TTGAGCCCGA GAGCATTAAC AGGTTAGTCC
3301 TGAACGGGGT GTTTGACATG ACCATGAAGA TCTGGAAGGT GCTGAGGTAC GATGAGACCAG
3361 GCACCAAGTG CAGACCCTGC GAGTGTGGCG GTAAACATAT TAGGAACCCG CTGTGTATGC
3421 TGGATGTGAC CGAGGAGCTG AGGCCCGATC ACTTGGTGCT GGCCTGCACC CGGCTGTAGT
3481 TTGGCTCTAG CGATGAAGAT ACAGATTGAG GTACTGAAAT GTGTGGCGCT GGCCTAAGGG
3541 TGGGAAGAA TATATAAGGT GGGGGTCTTA TGTAGTTTTG TATCTGTTTT CGACGACCGC
3601 CCGCGCCCAT GAGCACCAAC TCGTTTGATG GAAGCATTTG GAGCTCATAT TTGACAACGC
3661 GCGTCCCCC ATGGGCGGGG GTGCGTCAGA ATGTGATGGG CTCACGACTT GAGGTGCGCC
3721 CCGTCTGACC GCAAACTCT ACTACTTGA CTTGACGAGC CGTGTCTGGA AGCTGGTATGG
3781 AGACTGTGAC CTCGCGCCGC GCTTACGCGC CTGCAGCCAC CGCCCGCGGG ATGTGTAGCT
3841 ACTTGGTCTT CTTGAGCCGC TTTGCAAGCA GTGCAGCTTC CGGTTCACTC CGCCCGGATG
3901 ACAAGTGCAC GGTCTTTTTG GCACAATTGG ATCTTTGAC CGGGGAACCT AATGTGCTTT
3961 CTCAGCAGCT GTTGGATCTG CCCCAGCAGG TTTCTGCCCT GAAGGCTCCA TCCCCTCCA
4021 ATCGGGTTTA AAACATAAAT AAAAAACCAG ACCTGTGTTG GATTTGGATC AAGCAAGTGT
4081 CTGTGCTGCT TTATTTAGGG GTTTTGGCGC CCGGTAAGCG CCGGGACCAAC CGGTCTCGGT
4141 CGTTGAGGGT CTTGTGATT TTTTCCAGGA GTGACTCTGG GTGACTCTGG TATGTCAGAT
4201 ACATGGGCAT AAGCCCGTCT CTGGGGTGGA GGTAGCACCA CTCGAGAGCT TCATGTCTGG
4261 GGGTGGTGT TTAGATGATC CAGTCTGTAG AGGAGCGGTG GCGGTGTGTC CTAAAAATGT
4321 CTTTCAGTAG CAAGCTGATT GCCAGGGGCA GGCCCTTGCT GTAAGTGTGT ACAAGCCGGT
4381 TAAGCTGGGA TGGGTGCATA CGTGGGGATA TGAGATGCAT CTTGGACTGT ATTTTATAGT
4441 TGCTATGATT CCCAGCCATA TCCCTCCGGG GATTCACTGT GTGCAGAAC ACCAGCACAG
4501 TGTATCCGGT GCACITGGGA AATTGTGATC GTAGCTTAGA AGGAAATGCG TGGAAAGACT
4561 TGGAGACGCC CTTGTGACCT CCAAGATTTT CCATGCATTC GTCCATAATG ATGGCAATGG
4621 CCCCAGCGCG CGCGCGCTGG CGGAAGATAT TTCTGGGATC ACTAACGTCG TATGTGTGTT
4681 CCAGAGTAG ATCGTCTAG GCCATTTTTA CAAAGCGCGG CGGAGGGGTG CCAGACTCGG
4741 TCAATAGTGT TCCATCCGGC CCAGGGGCGT AGTTACCCTC ACAGATTTGC ATTTCCACAG
4801 CTTTGAAGTC AGATGGGGGG ATCATGTCTA CTGCGGGGGG GATGAAGAAA ACGGTTTCCG
4861 CGGTAGGGGA GATCAGCTGG GAAGAAAGCA GGTTCCTGAG CAGCTGCGAC TTACCGCAGC
4921 CCGTGGGCCC GTAAATCACA CTTATTACCG GTGCTCAACT GTAGTTAAGA GAGCTGCAGC
4981 TGCCGTCATC CTTGAGCAGG GGGGCCACTT CGTTAAGCAT GTCCCTGACT CCGATGTTTT
5041 CCGTGCACAA ATCCGCCAGA AGGCGCTCGC CGCCACGGA TAGCATTTCT TCGAAGGAAG
5101 CAAAGTTTTT CAACGGTTTG AGACCGTCCG CTGTAGGCAT GCTTTTGAAG TTTGACCAA
5161 CGAGTTCCAG CGGCTCCAC AGCTCGGTCA CCGTCTAC GGCATCTGGA TCGTGCATAT
5221 CTCCTGTTT CGCGGGTTGG GCGGGCTTTC GCTGTACGGC AGTAGTCGGT GCTCGTCCAG
5281 ACGGGCCAGG GTCATGTCTT TCCACGGGGG CAGGGTCTCT GTACGGGTGAG GTCCGGTTCG
5341 GGTGAAGGGG TGGCGTCCGG GCTGCGCGCT CGCTCGCGCT CGCTGTAGGC TGGTCTAGCT
5401 GTGCTGAAG CGTGCCTGGT CTTGCGCGCT CGCGTGGCG AGGTAGACTT TGACCAATGT
5461 GTCATATGTC AGCCCCCTCG CGGCGTGGCC CTTGCGCGCG AGCTTGCCCT TGGAGGAGCG
5521 CCGCAACGAG GGGCAGTGCA GACTTTTGAG GCGCTAGAGC TTGGGCGCGA GAAATACGGA
5581 TTTCCGGGAG TAGGCATCCG CGCCGACAGC CCGCGACAGG GTCTGCTATT CGACGAGCCA
5641 GGTGAGCTCT GCGCGTTCGG GGTCAAAAAC CAGGTTTCCC CATGTCTTTT TGATGGTGTG
5701 CTCTACCTCT GTTTCCATGA GCCGGTGTCC AGCTCGGTG ACGAAAGAGG TGTCCGTGTG
5761 CCGCTATACA GACTTGAGAG GCCTGTCTCT GAGCGGTGTT CGCGGTGCTC CTTGCTATGT
5821 AAACCTCGAC CACTCTGAGA CAAAGGCTCG GTCCAGGCG CTGACGAGG AGGCTAAGTG
5881 GGAGGGGTAG CGGTGTTGT CCACTAGGGG CCGTACTCGC TCCAGGGGTG GAAGACACAT
5941 GCGTCCCTCT TCGGCTCAA GGAAGGTGAT TGGTTTGTAG GTGTAGGCCA CTTGACCGGG
5901 TGTTCCTGAA GGGGGCTAT AAAAGGGGGT GGGGGCGCGT TCGTCTCAC CTGTCTCCG
6061 ATCGCTGTCT CGGAGGGCCA GCTGTTGGGG TGAAGTACTC CTCTGAAAAG CCGGCATGAC

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6121 TTCTGCGCTA AGATTGTCAG TTTCCAAAAA CGAGGAGGAT TTGATATTCA CTTGCCCGCG  
 6181 GGGTAGTGCT TTGAGGGTGG CCGCATCCAT CTGGTCAGAA AAGACAATCT TTTTGTGTCT  
 6241 AAGCTTGGTG CCAACGACCC CGTAGAGGGC GTTGACAGCG AACTTGGCGA TGGAGCGCAG  
 6301 GGTTTGGTTT TTGTGCGCAT CGGCGCGCTC CTGGCGCGCG ATGTTTAGCT GCACGTATTCT  
 6361 GCGCGCAACG CACCGCCATT CGGGAAGAAC GGTGGTGCGC TCGTCGGGCA CAGGTTGCAC  
 6421 GCGCCAAACG CGGTTGTGCA GGGTGACAAG GTCAACGCTG TGGGTACCTT CTCCCGGTAG  
 6481 GCGCTCGTTG GTCCAGCAGA GCGGCGCGCC CTGCGCGAG CAGAATGGCG GTAGGGGGTC  
 6541 TAGCTCGCTC TCGTCCGGGG GGTCTGCGTC CACGGTAAAG ACCCCGGGCA GCGGCGCGCG  
 6601 GTCGAAGATG TCTATCTTGC ATCCTTGCMA GTCTAGCGCC TGTCTGCTAG CGCGGCGCGC  
 6661 AAGCGCGCGC TCGTATGGGT TGAGTGGGGG ACCCCATGGC ATGGGGTGGG TGAGCGCGGA  
 6721 GGGGTACATG CCGCAATGT CGTAAACGTA GAGGGGCTCT CTGAGTATTCT CAGATATGTT  
 6781 AGGGTAGCAT CTTCCACGCG GGTATGCTGG CGCAGCTAA TCGTATAGTT CGTCCGAGGG  
 6841 AGCGAGGAGG TCGGAGCCGA GGTGTGCTAG GCGGGGCTGC TCTGCTCGGA AGACTATCTG  
 6901 CCTGAAGATG GCATGTGAGT TGGATGATAT GGTGTGACCG TGAAGAGCTT TGAAGCTGCG  
 6961 GTCTGTGAGA CTTACCGCGT CACGCACGAA GAGGCGTAG GAGTGCGCBA GCTTGTGTAC  
 7021 CAGCTCGGGG GTGACCTGCA CGTCTAGGGC GCAGTAGTCC AGGGTTTCTT TGATGATGTC  
 7081 ATACTTATCC TGTCCTTTT TTTTCCACAG CTCGCGGTG AGGACAAAT CTTCGCGGTG  
 7141 TTTTACGATG TCTTGGATCG GAAACCCGTC GCCTCCGAA CGGTAAAGAGC CAGTACGATG  
 7201 GAATCTGGTT AGCGGCTGGT AGGCGCAGCA TCCTTTTCTT ACGGGTAGCG GGTATGCTTG  
 7261 CGCGGCTTTC CGGAGCGAGG TGTGGGTGAG CGCAAGAGTG TCCCTGACCA CGATTTTGA  
 7321 GTACTGGTAT TTGAAGTCAG TGTCTGCGCA TCCGCGCTGC TCCGAGAGCA AAAAGTCCCT  
 7381 GCGCTTTTGG GAACCGGATG TTGGCAGGGC GAAGGTGACA TCGTTTGGCC GTATCTTTTG  
 7441 CGCGCGAGGC ATAAAGTTGC GTGTGATGCG GAAGGGTCCC GGCACCTCGG AACGGTGTGT  
 7501 AAATTAACCT GCGGCGAGCA CGATCTCGTC AAAGCGTTTG ATGTTTGGGC CCACATGTAT  
 7561 AAGTTCOAAG AAGCGCGGGA TGCCCTTGAT GGAAGGCAAT TTTTATAGTT CTCTGTAGGT  
 7621 GAGTCTTCTA GCGGAGCTGA GCCCTGCTTC TGAAGGGGCC CAGTCTGCAA GATGAGGGTT  
 7681 GGAAGCGCAG AATGAGCTCC ACAGGTCAAG GGCATTAGC ATTGTGCAGT GGTGCGAAAA  
 7741 GGTCTTAAC TGCGACCTTA TGGCAATTTT TGTGSGGTG ATGCAAGTAGA AGTAAAGCGG  
 7801 GTCTTGTGTC CAGCGGTCCC ATCCAAGGTT CCGCGCTAGG TCTCGCGCGG CAGTCACTAG  
 7861 AGGCTCATCT CCGCCGAATC TCATGACGAG CATGAAGGGC ACAGACTGCT TCCCAAGGCT  
 7921 CCCCATCCAA GTATAGGTTCT CTACATCGTA GGTGACAAAG AGACGCTCGG TCGAGGATG  
 7981 CGAGCGCATC GGAAGAATCT GGATCTCCCG CACCAATATG GAGGAGGTGC TATTGATGTG  
 8041 GTGAAAGTAG AAGTCCCTGC GACGGGCGGA ACACTCGTGC TGGCTTTTGT AAAAAGCTGC  
 8101 CGAGTACTGG CAGCGGTGCA CGGCGCTGAT ATCCTGCACG AGGTGCACTT GACGACCGCG  
 8161 CACAAGGAAG CAGAGTGGGA ATTTGAGCCC CTGCGTGGC GGGTTTGGCT GGTGCTCTTC  
 8221 TACTTGGCTT GCTTGTCTTT GACGCTCTGG CTGCTCGAGG GGAAGTTACG TGGATCGGAG  
 8281 CACCAACGCG CGCGAGCCCA AAGTCCAGAT GTCCGCGCGC GCGGTCGGA GCTTGTGAC  
 8341 AACATCGCGC AGATGGGAGC TGTCCATGGT CTGAGCTTCC CGCGCGCTCA GGTCAAGCGG  
 8401 GAGCTCTTGC AGGTTTACCT CGCATAGACG GGTCAAGGCG CGGCTAGATG CCAGGTGATA  
 8461 CCTAATTTCC AGGGGCTGGT TGTGTGCGCG GTGATGGCT TGCAGAGAGC GCATCCCCG  
 8521 CGGCGGACTC ACGGTACCGC GCGGCGGGCG GTGGGCGCGG GGGGTGCTCT TGGATGATG  
 8581 ATCTAAAGCG GGTGACCGCG GCGAGCCCCC GAGGATAGGG GGGGCTCGCG ACCCGCGGGG  
 8641 AGAGGGGGCA GGGCAAGCTC GCGGCGCGCG GCGGCGAGGA GCTGTGCTCT TGGATGATG  
 8701 TTGCTGGCGA ACAGCAAGAC CGCGCGGTTG ATCTCTGAA TCTGGCGCTT CTGCGTGAAG  
 8761 ACAGCGGGCC CGGTGAGCTT GAGGCTGAAA GAGAGTTCGA CAGAATCAAT TCTGGTGTGG  
 8821 TTGACGGCGG CCGTGGCGAA AATCTCTCTG ACGTCTCTCT AGTTGTCTTG ATAGGCGATC  
 8881 TCGGACATGA ACTGCTCGAT CTCTTCTCTT TGGAGATCTC CGCTCCCGG CTCTCCAGC  
 8941 GTGGCGGAGC GGTGTTTGA GATGCGGGCC AATGCGGGCC CTTTCTGAGT CCGCGGCGCG CAGCTGAAG  
 9001 TCGTTCCAGA CCGGCTGTGA GACCAACGCC CTTTCTGAGT CGCGGGCGCG CATGACGAGC  
 9061 TCGCGGAGAT TGAGCTCCAC GTGCGGGGCG AAGACGGCGT AGTTTGGCAG CGCTGGAAG  
 9121 AGGTAGTTGA GGTGTGTGCG GGTGTGTTCT GCGCAAGAAC AGTACATATC CAGCGCTGCT  
 9181 AACGTGGATT CGTTGATATC CCCCAGGGCC CCGGACAGCG TTAATCTCTC CTCCAGAGA  
 9241 ACGGCGAAGT TGA AAAAAGT GGAAGTTGCG GTGCGGACCT TCGCGCTCAA AGGCTACAGG GGCCTCTTCT  
 9301 CGGATGAGCT CGGCGACAGT GTGCGGACCT TCGCGCTCAA AGGCTACAGG GGCCTCTTCT  
 9361 TCTTCTTCAA TCTCTCTTCT CATAAGGGCC TCCCTCTTCT TCTTCTTCTG CGGCGGTGGG  
 9421 AGAGGGGGGA CACGGCGGCG ACAGACGGCG CATGCTCTCG GTGACGGCGC GGGCGTTTCT CGGCGGGCGC  
 9481 ATCTCCCGCG GCGACGGCG

FIGURE 21  
(SHEET 3)

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9541 AGTTGGAAGA GCGCCGCCGT CATGTCGCCG TTATGGGTTG GCGGGGGGCT GCCATCGCGC  
 9601 AGGGAATACGG CGCTAACGAT GCATCTCAAC AATTGTTGTG TAGGTACTCC GCCGCGAGG  
 9661 GACCTGAGCG AGTCCGCATC GACCCGGATCG GAAAACTCTC CGAGAAAGCG GTCTAACACG  
 9721 TCACAGTACG AACGGTAGGCT GAGCACCCTG CGCGCGCGCA CGCGCGCGCG GTCGGGGGTTG  
 9781 TTCTCGGCGG AGGTGCTGCT GATGATGTAA TTAAAGTAGG CGGTCTTAGG ACGGCGGGATG  
 9841 GTGACAGAAA GCACCATGTC CTGCGGTCGCG GCCTGCTGAA TGCGCAGGCG GTGCGGCAATG  
 9901 CCCCAGGCTT CGTTTGTACA TCGCGCGCAGG TCTTTGTAGT AGTCTTGAGT GAGCGTTTCT  
 9961 ACCGCGCACTT CTCTCTCTCC TTCTCTCTGT CCTGCACTCC TTGCACTATP CGCTGCGGCG  
 10021 GCGGCGGAGT TTGGCGGTAG GTGGCGCCCT CTCTCTGCCA CGCTGTATAT GCGCTGCTCG  
 10081 CTCATCGGCT GAAGCAGGCG TAGGTGCGCG ACAACGCGCT CGCTCAATAT TGCGCCCGTG  
 10141 ACCTGCGTGA GGGTAGACTG GAAGTCATCC ATGTCCACAA AGCGGTGGTA TGCGCCCGTG  
 10201 TTGATGGTGT AAGTGCAGTT GGCCATAACG GACCAGTTAA CGGTCTGGTG ACCCGGCTCG  
 10261 GAGAGCTCGG TGTACTGAG ACOCGAGTAA GCCCTCGAGT CAAATACGTA GTGTTGCAAA  
 10321 GTCCGCACCA GGTACTGGTA TCCACCAA AAAGTGCGGCG CGCGCTGGCG GTGCGAGGCG  
 10381 CAGCGTAGGG TGGCGGGGCG TCCGGGGGCG AGATCTTCCA ACATAAGGCG ATGATATCGG  
 10441 TAGATGTACC TGGACATCCA GGTGATGCGG CGCGCGGTGG TGGAGGCGTG CGGAAGCTCG  
 10501 CGGACGCGGT TCCAGATGTT GCGCAGCGCG AAAAAGTGCT CCATGTTGCG GAGCTCTCGG  
 10561 CCGGTGAGCG GCGCGCAATC GTTGACGTGCT TAGACGCTGC AAAAGAGAGG CACTGTAGAG  
 10621 GGCACTCTCT CGTGGTCTGG TGGATAAATT CGCAAGGGTA TCATGCGGGA CGACCGGGGT  
 10681 TGAGACCCCG TATCCGCGCG TCCGCGGTGA TCCATGCGGT TACCGCCGCG GTGTGCAAGC  
 10741 CAGGTGTGCG ACGTGACAGA ACGGGGGAGT GCTCTTTTGG GCTTCTTCCC AGGCGCGGCG  
 10801 GCTGCTGCGC TAGCTTTTTT GGCCACTGGC CGCGCGCAGG GTAAAGCGTT AGGCTGAGTG  
 10861 CGGAAAGCAT TAAGTGGCTC GCTCCCTGTA GCGCGAGGGT TATTTTCCAA GGGTGTAGTG  
 10921 GCGGGACCCC CGGTTGAGAT CTCCGACCGG CCGGACTGCG CGGAAAGCGG GTTTGCTCTC  
 10981 CGGTCATGCA AGACCCCGCT TGCAAAATTC TCCGGAACAA CGGACGAGCT CTTTTTTTCG  
 11041 TTTTCCGAGA TGATCCGGT GCTGCGGCG AGTCCGCCCC CTCTCTGAGA GCGCGAAGAG  
 11101 CAAGAGCAGC GGCAGACATG CAGGGCACCC TCCCCTCTCT CTACCGCGTC AGGAGGGGCG  
 11161 ACATCCGCGG TTGACGCGCG AGCAGATGGT GATTACGAAAC CCGCGCGGCG CCGCGCCCGG  
 11221 CACTACTCGG ACTTGGAGGA GGGCGAGGGC CTGCGCGGCG TAGGAGCGCC CTCTCTGAG  
 11281 CGGTACCCAA GGGTGCACT GAAAGCGTAT ACGCGTAGGG CGTACGTGCC CGCGCAGAAC  
 11341 CTGTTTTCGCG ACCCGAGGGG AGAGGAGGCC GAGGAGATGC GGGATCGAAA GTTCCAGCGA  
 11401 GGGCGCGAGC TGCGGCATGG CCTGAATCGC GAGCGGTTGC TGCGCGAGGA GGAATTTGAG  
 11461 CCGGACGCGC GAACCGGGAT TAGTCCCGCG CGCGCACACG TGCGCGCGCG CGACTCGGTA  
 11521 ACCGCAATCG AGCAGACGGT GAACCAAGGAG ATTAACCTTC AAAAAGGCTT TAACAAACCA  
 11581 GTGCGTAGCG TTGTGGCGCG CGAGGAGGTG GCTATAGGAC TGATGCATCT GTGGGACTTT  
 11641 GTAAGCGCGC TGGAGCAAAA CCCAAATAGC AAGCGGCTCA TGCGCGAGCT GTTCTCTATA  
 11701 GTGACGACCA GCAGGGACAA CGAGGCATTG AGGAGTGCGC TGCTAAACAT ATGAGAGGCC  
 11761 GAGGCGCGCT GGCTGCTCGA TTGTATAAAC ATCTCTGAGA GCATAGTGGT CGAGGAGCGC  
 11821 AGCTTAGGCC TGCTGACAAA GGTGGCGCGC ATCAACTATT CCATGCTTAG CTGTGGCAAG  
 11881 TTTTACGCCC GCAAGATATA CCATACCCTT TACGTTCCCA TAGACAAGGA GGTAAAGATC  
 11941 GAGGGGGTTCT ACATGCGCAT GCGCTGAGCT GTGCTTACTT TGAGCGACGA CCGTGGGCTT  
 12001 TATCGCAACG AGCGCATCCA CAAGCCCGTG ACGCTGAGCG GCGCGCGCGA GCTCAGCGAC  
 12061 CCGGAGCTGA TGCAACGCTT GCGAAAGGCG GTGCGCTGAG CTGCGTGCCA CCGCGACCGG CGATAGAGAG  
 12121 GCGGAGTCTT ACTTTGACCG GGGCGCTGAC CTGCGCTGGG CCCCAGCGCG ACGCGCCCTG  
 12181 GAGGCAAGCT GGGCGGAGC TGGGCTGGGG GTGCGACCGG CGCGCGCTGG CAAGCTCGGG  
 12241 GCGGTGAGAG AATATGACGA GGAAGATGAG CAACGGAGCC GCGGTGCGG GTACTAAGCG  
 12301 GTGATGTTCT TGATCAGATG ATGCAAGACG CAACGGAGCC GCGGTGCGG GTGACTAAGCG  
 12361 AGAGCGCAGC GTCCGCGCTT AACTCCACGG ACAGCTGCGG CCGGTGCGG CAGCGCATCA  
 12421 TGTGCTGAC TGCGCGCAAT CCTGACGCGT TCCGCGCAGG GCGCGAGGCC AACCGGCTCT  
 12481 CCGCAATTTT GGAAGCGGTG GTCCCGCGCG CCGCAAAACC CAGCGACGAG AAGGTTGCTG  
 12541 CGATCGTAAA CGCGCTGGCG GAAAAAGGGG CCATCCGCGC CGACGAGGCG GGCTGTGGTT  
 12601 ACAGACGCGCT GCTTCAAGCG GTGGCTCGTT ACAACAGCGG CAACGTGACG ACCAAGCTGG  
 12661 ACCGCTGTGT GGGGGATGTT CGCGAGGCGG TGGCGCAGCG TGAGCGCGCG CAGCAGCAGG  
 12721 GCAACCTGGG TCTCATGGTT GCACTAAAGC CCTTCTGAG TACACAGGCC GCGCACTGTC  
 12781 CCGCGGACGA GAGGACTAC ACCAACTTTG TGAGCGCACT GCGGCTAATG GTGACTGAGA  
 12841 CACCGCAAGT TGAGGTGTAC CAGTCTGGCG CAGACTATT TTTCAGAGCC GTAGCTAAGG  
 12901 GCCTGACGAC CGTAAACCTG AGCCAGGCTT TCAAAAATTG GCAGGGGCTG TGGGGGGTGC

FIGURE 21  
(SHEET 4)

12961 GGGCTCCAC AGGCGACCGC GCGACCGTGT CTAGCTTGCT GACGCCCAAC TCGCGCCTGT  
 13021 TGCTGCTGCT AATAGCGCCC TTCACGGACA GTGGCGAGCT GTCCCGGGAC ACATACCTAG  
 13081 GTCACTTGCT GACACTGTAC GCGGAGGCCA TAGGTCAAGC GCATGTGGAG GACGATACCT  
 13141 TCCAGGAGAT TACAAGTGTC AGCGCGCGCG TGGGCGAGGA GGACACGGGC AGCCTGTAGG  
 13201 CAACCTATA CTACCTGCTG ACCAACCGGC GGCAGAAGAT CCCCTGTTGT CACAGTTTAA  
 13261 ACAGCGAGGA GAGAGCGCAT TTGCGCTACG TGCAGCAGAG CGTGAGCCCT AACCTGATGC  
 13321 GCGAGCGGGT AACGCCACGC GTGGCGCTGG ACATGACCGC GCCTAAATGGA CTACTTGATC CGCGCGGCCG  
 13381 TGTATGCTCT AAACCGCGCC TTATCAACCC GCCTAAATGGA CTACTTGATC CGCGCGGCCG  
 13441 CGGTGAACCC CGAGTATTTT ACCAATGGCA TCTTGAACCC GCATGTGCTC CGCGCGGCCG  
 13501 GTTGTACACG CGGGGATTC GAGGTGCCCG AGGGTGAACG TGAGTTGCTC GACAGCGACA  
 13561 TAGACGACAG CGTGTTTTCC CCGCAACCGC AGACCCCTGT AGAGTTGCA CAGCGCGAGC  
 13621 AGGCGAGGCG GCGCGTGGCA AAGGAAAGCT TCOCGAGGCC AAGCAGCTTG TCCGATCTAG  
 13681 GCGCTGCGCG CCGCGGGTCA GATGCTAGTA GCCCATTTCC AAGCTTGATA GGGTCTCTTA  
 13741 CCAGACTCG CACCAACCGC CCGCGCTGCG TGCGCGAGGA GGAGTACTTA AACCACTCGC  
 13801 TGCTGACGCC GCAGCGCGAA AAAAACCTGC CTCCGCGATT TCCCAACAC GGGATAGAGA  
 13861 GCCTAGTGGA CAAGATGAGT AGATGGAAGA GTACGCGCA GGAGCAGAG GACGTGCCAG  
 13921 GCGCGCGCCC GCCACCCGCT CGTCAAAGGC ACGACCGTCA CGCGGGTCTG GTGTGGGAGG  
 13981 ACGATGACTG GCGAGACGAC AGCAGCGTCC TGGATTGGGG AGGAGTACCA AACCCGTTTG  
 14041 CGCACTTCAG CCCAGGCGTG GGGAGATGAT TTAAAAAATA AAAAGACATG ATGCAAAATA  
 14101 AAAAATCAC CAAGGCCATG GCACCGAGCG TTGTTTTTCT TGTATTCCTC TTAGTATGCG  
 14161 GCGCGCGCGC ATGTATGAGG AAGTCTCTCC TCCTCTCTAC GAGAGTGTGG TGAGCGCGGC  
 14221 CGCACTGGCG GCGCGCTGG GTTCTCCCTT CGATGCTCCC CTGGACCTGG CTTTGTGGCC  
 14281 TCOCGGGTAC CTGCGGCTA CCGGGGGGAG AAACAGCATC CGTACTCTCT AGTTGGCAC C  
 14341 CCTATTTCAG ACCAACCGTG TGTACCTGGT GGACAACAAG CGTACTCTCT AGTTGGCAC C  
 14401 GAATACACAG AACGACACCA GCAACTTTCT GACCAACGGT ATTTCAAACA ATGACTACAG  
 14461 CCGCGGGGAG GCAAGCACAC AGAACATCAA TCTTGACGAC CGGTGCGACT GGGCGGGGAA  
 14521 CCGTAAAAAC ATCTGCGATA CCAACATGCC AAATGTGAAC GAGTGTCACT TTACCAATTA  
 14581 GTTTAAGGCG CGGTGTATGG TGTCGCGCTT GCCTACTAAG GACATACAGG TGAGGCTGAA  
 14641 ATACGAGTGG GTGGAGTTCA CGCTGCGCGA GGGCACTAC TCCGAGACGA TGACCATAGA  
 14701 CCTTATGAAC AACCGGATCG TGGAGCACTA CTTGAAAGTG GGCAGACAGA ACGGGGTTCT  
 14761 GGAAGGCGAC ATCGGGGTAA AGTTTGACAC CCGCAACTTC AGACTGGGGT TTGACCCCGT  
 14821 CACTGGTCTT GTCAATGCTG GGGTATATAC AAACGAAGCC TTCCATCCAG ACATCATTTT  
 14881 GCTGCGAGGA TCGGGGGTGG ACTTCAACCA CAGCGCGCTG AGCAACTTTG TGCGCATCCG  
 14941 CAAGCGGCAA CCTTCCAGG AGGGCTTTAG GATCACTAC GATGATCTGG AGGGTGGTAA  
 15001 CATTTCCGCA CTGTTGGATG TGGACGCTTA CAGGCGGAGC TTGAAAGATG ACACCGAACA  
 15061 GGGCGGGGGT GGGCGAGGCG GCAGCAACAG CAGTGGCAGC GGGCGGAGAG AGAATCTCAA  
 15121 CGCGGCGGCG CGGCGAATGC AGCGGTGGA GGCATGAAC GATCATGCA GATCGCGGA  
 15181 CACTTTTGCT ACACGGGCTG AGGAGAAGCG CGCTGAGGCC GAAGCAGGCG CCGAGATGTC  
 15241 CGCCCGCGCT CGCAACCGC AGGTGAGAA GCGTCAAGAG CCTCAGAAG AAACCGGTGA TCAAACCTCT  
 15301 GACAGAGGAC AGCAAGAAAC GCAATTACAA CCTAATAAGC AATGACAGA CCTTCAACCA  
 15361 GTACCGCAGC TGTGACTCTG CATACAACCTA CGCGACCCCT CAGACCGGAA TCCGCTCATG  
 15421 GACCCCTGCT TGCACTCTG ACGTAACTCT CGGCTCGAG CAGGTCTACT TGTGCTTGCC  
 15481 AGACTGATG CAGAGCCGCG TGACCTTCGCT CTCACGCGC CAGATCAGCA ACTTCCGGT  
 15541 GGTGGCGGCC GAGCTGTTCG CGGTGCACTC CAGAGGCTTC TACAACGACC AGGGCTGCTA  
 15601 CTCCCACTC ATCCGCCAGT TTACTCTCTG GACCACGCTG TTCAATGCTT TTCCCGAGAA  
 15661 CAGATTTTTG GCGCGCCGCG CACGCCCAAC CATCAACCA GTCAGTGAAG ACGTGTCTGC  
 15721 TCTCAGAGT CAGCGGACGC TACCGCTGCG CAACAGCTAT GAGAGGATGC AGCGAGTGAC  
 15781 CATTAAGTAC GCGAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GACGATCTCT  
 15841 GCGCGCGGTC CTATCGAGCC GCACCTTTTG AGCAAGCATG TCCATCTTTA TATCGCCAG  
 15901 CAATACACAA GGGTGGGGCC TGCGCTTCCC TGCGCTGCGG AACCAAGAGC CCAAGAGAGC  
 15961 CTCCGACCAA CACCGAGTGC GCGTGGCGGG GCACTACCGC GCGCCCTGGG GCGCGCACAA  
 16021 ACGCGCGCGC ACTGGGCGCA CCAACGTCGA GACGCGCATC GACGCGGCGA TTAGACCGGT  
 16081 GCGCAACTAC ACGCCACGCG CGCCACCACT GTCCACAGTG GACGCGGCGA TTAGACCGGT  
 16141 GGTGCGCGGA GCGCGCGCTG ATGCTAAAT GAAGAGACGG CCGAGAGCGC TAGCAGCTG  
 16201 CCACCGCGCG GACCGCGGCA CTGCGGCCCA ACGCGCGCGC CGCGCCCTGC TTAGACCGGT  
 16261 ACGTGCACCC GCGCGAGCG GCGCATGCG GCGCGCTCGA AGGCTGTCGG CGGGATTTGT  
 16321 CACTGTGCCC CCCAGGTCCA GCGCACGAGC GGCUCGCGCA GCAACCGCGC CCATTAGTGC

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16381 TATGACTCAG GGTGCGCAGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCGCTGG  
 16441 CGTGCCCGTG CGCACCCGCG CCCCGCGCAA CTAGATTGCA AGAAAAAAT ACTTAGACTC  
 16501 GTACTGTTGT ATGTATCCAG CGCGGGCGCG GCTATGTCCA GCTATGTCCA AGCGCAAAAT  
 16561 CAAAGAGAGC ATGTCTCCAG TCATCGCGCG GGAGATCTAT GGCCCCCGGA AGAAGGAAGA  
 16621 GCAGGATTAC AAGCCCCGAA AGCTAAAGCG GTCAAAAAGG AAAAAGGAAG ATGATGATGA  
 16681 TGAATTTGAC GACGAGGTGG AACTGCTGCA CGCTACC CGC CCCAGGCGTAC GGGTACAGTG  
 16741 GAAAGGTGCA CGCTAAAAAC GTGTTTGGG ACCCGGCACT ACCGTAGCT TTACGCCCGG  
 16801 TGAGCGCTCC ACCCGCACTC ACAAGCGCGT GTATGATGAG GTGTACGCGG ACGAGGACCT  
 16861 GCTTGAACAG GCCAACGAGC GCCTCGGGGA GTTTCCTAC GGAAGCGCGC ATAGAGACAT  
 16921 GCTGGCGTTG CGCTGAGAGC AGGCGCAACCC AACACCTAGC CTAAGCGCGC TAACAGTCGA  
 16981 GCAGGTGCTG CCCGCGCTTG CACCGTCCGA AGAAAAGCGC GGCTAAAGC GCGAGTCTGG  
 17041 TGACTTGCA CCCACCGTGC AGCTGATGGT ACCCAAGCGC CAGCGACTGG AAGATGTCTT  
 17101 GGAATAAATG ACCGTGGAAC CTGGGCTGGA GCCCGAGGTC CGCGTGGCGC CAATCAAGCA  
 17161 GTTGGGCGCG GGACTGGGCG TGCAAGCCGT GGACGTTTCA ATACCCACTA CCAGTAGTAC  
 17221 CAGTATTGCC ACCGCCACAG AGGGCATGGA GACACAAAGC TCCCGGTTG CCTCAGCGGT  
 17281 GCGGATGCC GCGGTGCAGG CGGTGCTGCG GCGCGCGTCC AAGACCTCTA CGGAGGTGCA  
 17341 AACGGACCCG TGGATGTTTT GCGTTTCAGC CCCCCGCGC CCGCGCGGTG CGGAGTAGTA  
 17401 CGCGCGCGCC AGCGCGCTAC TGCCCGAATA TGCCCTACAT CTTCTCACTT CGCTACCC  
 17461 CGGCTATCGT GGTACACCT ACCGCCCCAG AAGACGAGCA ACTACCCGAC GCGCAACACG  
 17521 CACTGGAACC CGCGCGCGCG GTCGCGCTCG CCAGCCCGTG TGTGCCCCGA TTTCCTGGG  
 17581 CAGGGTGCTC CGCGAAGGAG GCAGGACCCCT GGTGCTGCCA ACAGCGCGCT ACCACCCGAC  
 17641 CATCGTTTAA AAGCCGGTCT TTGTGTTCT TGCAAGATAT GCCTCAGCT CGCGCTCCG  
 17701 TTTCCCGGTG CGCGGATTCC GAGGAAGAAT GCACCGTAGG AGGGCGTAGG CCGGCGCACG  
 17761 CTGACGCGCG GGCATGCGTC GTGCGCACCA CCGGCGCGCG CGCGCTGTCG ACCGTGCTAT  
 17821 CGCGCGCGGT ATCTCGCCCC TCCTATTTC ACTGATCGCC GCGGCGATGT GCGCGCTGCC  
 17881 CGGAATTGCA TCCGTGSCCT TGCAAGCGCA GAGACACTGA TTAATAACAA GTTGATGTGT  
 17941 GAAAAATCAA AATAAAAAGT CTGAGCTCTC AGCTCGCTT GGTCTGTGAA CTATTTTGTA  
 18001 GAATGGAAGA CATCACTTT GCGTCTCTGG CCCCAGACA CGGCTCAGCG CGGTCTATGG  
 18061 GAACTGCGCA AGATACTGCG ACCAGCAATA TGAGCGGTGG CGCTTTCAGC TGGGCTCGC  
 18121 TGTGAGCGCG CATTAATAAT TTGCGTTCCA CCGTTAAGAA CTAATGCGAC AAGCGCTGGA  
 18181 ACAGCAGCAC AGGCGCAGTG CTGAGGAGTA AGTTGAAGA GCRAAAATTC CAACAAAAGG  
 18241 TGGTAGATGG CTTGGCTCTT GGCATTAGCG GGGTGTGGA CCTGGCCAC CAGGACGTGC  
 18301 AAAATAGATG TAACAGTAAG CTTGATCCCG GCCTCCGCT AGAGGAGCCT CCACGGCGCG  
 18361 TGGAGACAGT GTCTCCAGAG GGGCGTGGCG AAAAGCGTCC GCGCGCCGAC AGGGAAGAAA  
 18421 CTCTGTTGAC GCAAAATAGC GAGCCTCCCT CGTACGAGGA GGCACTAAGC CAAGGCTTGC  
 18481 CCACACCCCG TCCCATCGCG CCGATGCTTA CGGAGTGCT GGGCGAGCAG ACACCGGTAA  
 18541 CGCTGAGACT GCTTCCCGCC GCGGACACCC AGCAGAAACC TGTGCTGCCA GCGCCGACCG  
 18601 CGGTGTTGT AACCCTCTCT AGCCCGCGCT CCTTCCGCG CGCCGCGCAG GTTCCGCGAT  
 18661 CGTTGCGCGC CGTAGCCAGT GGCAACTGGG AAGCAGCACT GAACAGCAGT GTGGTCTGG  
 18721 GGGTGCAATC CTTGAAGCGC CGACGATGCT TCTGAATAGC TAACGTGTGC TATGTGTGTC  
 18781 ATGTATGGGT CCATGTGCGC GCGAGGAGG CTGCTGAGCG GCGCGCGCGC GCTTTCCAA  
 18841 GATGGCTACC CTTTCGATGA TGCGCGCAGTG GTCTTACATG CACATCTCGG GCCAGGAGCG  
 18901 CTGCGGATGC CTGAGCCCGG GGCTGGTGCA GTTTGCGCCG GCCACCGAGA CGTACTTCAG  
 18961 CCTGAATAAC AAGTTTAGAA ACCCCACCGT GGGCGCTAGC CAGACAGTGA CCACGAGCG  
 19021 GTCCACAGGT TTGACGCTGC GGTTCATCCG TGTGAGCCGT GAGGATGCT GTACTCTGTA  
 19081 CAAGGCGCGG TTACCCCTAG CTGTGGGTGA TAACCGTGTG CTGAGACGTA GTTCCACGTA  
 19141 CTTTGAATC CGCGCGGTGC TGGACAAGGG CCTACTTTT AAGCCCTACT GCACTACTGC  
 19201 CTACACCGCC CTGGCTCCCA AGGCTGCGCC AATCTTTTC GAATGSGATG AAGCTGCTAC  
 19261 TGCTCTTGA AATAACCTAG AAGAAGAGGA AGATGACAC GAAGACGAGT TAGACAGCA  
 19321 AGCTGAGCAG CAAAAAATC ACGTATTGCG GCGAGCGCCT TATTCTGCTGA TAAATATTAT  
 19381 AAGAGAGGGT ATTCAATAG GTGTGCAAGG TCAAAACACT AAATATGCGC ATAAATACCT  
 19441 TCAACCTGAA CCTCAATAG GAGATCTCA GTGGTACGAA ACTGAAATTA ATCATGACG  
 19501 TGGGAGAGCT CTTAAAAAGA CTACCCCAAT GAAACCATGT TACGGTTTAT ATGCAAAACC  
 19561 CAAATGAAA AATGGAGGCG AAGGCATTCT TGTAAAGCAA CAAAATGAGG ATGTAGAAAG  
 19621 TCAAGTGGAA ATGCAATTTT TCTCAACTAC TGAGGCGACC GCGAGGCATG GTGATACCT  
 19681 GACTCTTAAA GTGTATTGT ACAGTGAAGA TGTAGATATA GAACCCCAAG ACACCTCAT  
 19741 TTCTTACATG CCACTATTA AGGAAGGTAA CTCACGAGAA CTAATGGGCG AACAATCTAT

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19801 GCCAACACAGG CCTAATTACA TTGCTTTTAG GGACAATTTT ATTGGTCTAA TGTATTACAA
19861 CAGCAGCGGTG AATATGGGTG TTCTGGCGGG CCAAGCATCG CAGTGAATAG CTGTGTGTAG
19921 TTGCAACAGAC AGAAACACAG AGCTTTTCATA CCAGCTTTTG CTGTGATGCC TTGTGTGTAG
19981 AACCCAGGTAC TTTTCTATGT GGAATCAGGC TGTGTACAGC TATGATCCAG ATGTGTAGAAT
20041 TATTGAAATAT CATGGAACCT AAGATGAACCT TCCAATATAC TGCTTTCCAC TGCGAGGGTGT
20101 GATTAAATACA GAGACTCTTA CCAAGGTAAA ACCTAAACAA GGTCAGGAAA ATGTGTGGGA
20161 AAAAGATGCT ACAGAAATTTT CAGATAAAAA TGAATAAAGA TTGTGGAAAT ATTTTGGCAT
20221 GGAAATCAAT CTAAATGCCA ACCTGTGGAG AAATTTTCGT TACTTCAACA TAGCGCTGTG
20281 TTGGCCCGAC AAGCTAAAGT ACAGTCTCTT CAACGTAATA ATTTCTGATA ACCCAAACAC
20341 CTACGACTAC ATGAACAAGC GAGTGGTGCC TCCCGGGTTA GTGGACTGTG GTCTTAACCT
20401 TGGAGCACGC TGGTCCCTTG ACTATATGGA CAACGTCAC CCATTTAACC ACCACCGCAA
20461 TGCTGGCGTG CGCTACCGCT CAATGTTGCT GGGCAATGGT CGCTATGTGC CCTTCCACAT
20521 CCAAGTGCTCT CAGAAGTTCT TTGCCATTAA AAACCTCTCT CTCTCGCGG GCTCATACAC
20581 CTACGAGTGG AACTTCAGGA AGGATGTTAA CATGGTCTCT CAGAGCTCCC CTGGAATGA
20641 CCTAAGGGTT GACGGAGCCA GCATTAAAGT TGATAGCATT TGCCCTTACG CCACCTTCTT
20701 CCCCATGGCC CACAACACCG CTTCCAAGCT TGAGGCCATG CTTAGAAACG ACACCAACGA
20761 CCAGTCTCTT AACGACTATC TCTCCGCGC CAACATGCTC TACCCTATAC CGCGCAACGC
20821 TCCAAACGTG CCCATATCCA TCCCTCTCCG CAACGTGGGT GCTTTCGGG CTCTGGGCTT
20881 CACCGCGCTT AAGACTAAGG AAACCCATC ACTGGGCTCG GGCTACGAC CTATTATGAC
20941 CTACTCTGGC TCTATACCT ACCTAGATGG AACCTTTTAC CTCAACCAAC CTTTAAAGAA
21001 GGTGGAGATT ACCTTTGACT CTCTGTCTAG CTGGCTGGC AATGACCGCC TGCTTACCOC
21061 CACGAGTTT GAAATTAAGC GCTCAGTTGA CGGGGAGGGT TACAACGTTG CCGATGTATA
21121 CATGACCAAA GACTGOTTTT TGGTACAAAT GCTAGCTAAC TACAACATTG GCTTACCAGG
21181 CTCTTATATC CCAGAGAGCT ACAAGGACCG CATGTACTCT TTCTTTAAGA CCTTCCAGCC
21241 CATGAGCGCT CAGGTGGTGG ATGATACTAA ATACAAAGAC TACCAACAGG TGGCATCTCT
21301 ACACCAACAC AACAACTCTG GATTTGTTGG CTACCTTGCC CCCACATGC GCGAAGGACA
21361 GGCCTACCTT GCTAACTTCC CCTATCCGCT TATAGGCAAG ACCCGAGTTG ACAGCATTTAC
21421 CCGCAAAAAG TTTCTTTCCG ATCGCACCTT TTGGCGCATC CCATTCCTCA GTAACTTTAT
21481 GTCCATGGGC GCACCTACAG ACCTGGGCCA AAACCTTCTC TAGCCCAACT CGGCCACGCG
21541 GCTAGACATG ACTTTTGAGG TGGATCCCAT GGACGAGGCC ACCCTTCTTT ATGTTTGTGT
21601 TGAAGTCTTT GAGCTGGTCC GTGTGCACCG CGCGCACCGC GCGCTCATCG AAACCGTGTG
21661 CCTGCGCACG CCTTCTTCGG CGGCAACGCG CACAACATAA AGAAGCAAGC AACATCAACA
21721 ACAGCTGGCG CCATGGGCTC CAGTAGAGCA GAACGTGAAAG CCATTGTCAA AGATCTTGGT
21781 TGTGGGCCAT ATTTTITGGG CACCTATGAC AAGCGCTTTC CAGGCTTTGT TTCTCCACAC
21841 AAGTTCGCTT GCGCCATAGT CAATACGGCC GGTGCGGAGA TCGGGGGGCT ACATGGATG
21901 GCTTTTGCTT GGAACCCGCA CTCAAAAACA TGCTACTCTT TTGAGCCCTT TGGCTTTTCT
21961 GACCAAGGAC TCAAGCAGGT TTACCAAGTT GAGTAGCAGT CACTCCTCGG CGGTAGCGCC
22021 ATTGCTTCTT CCCCGACGCG CTGTATAACG CTGGAAGAGT CCACCCAAAG CGTACAGGGG
22081 CCAACTCTGG CGCGCTGTGG ACTATTCTCG TGCATGTTCT TCCAAGCTTG TCCCAACTGG
22141 CCCCAACTCT CCATGGATCA CAACCCACCC ATGAACCTTA TTACGGGGGT ACCCAACTCC
22201 ATGCTCAACA GTCCCCAGGT ACAGCCACCC CTGCGTGCAG ACCGAGAACA GCTCTACAGC
22261 TCTTGGAGCG GCCACTCGCC CTACTTCCG AGCCACAGT GCGCAGATTAG GAGCGGCATC
22321 TTCTTTGTGC ACTTGA AAAA CATGTAAAAA TAATGTACTA GAGACAGATT CATTAAGAGC
22381 AATGTGCTTT AITTGTACAC TCTCGGGTGA TTAATTACCC CCACCTTGC GCTCTCGGCC
22441 GTTTAAAAAT CAAAGGGGTT TCGCCGCGCA TCGCTATGCC CACTCCGCGG GACAGCTTGG
22501 CGATATCGGT GTTTAGTGTCT CCACTTAAAC TCAGGCACAA CCATCCCGGG CAGCTCGGTG
22561 AAGTTTTCAC TCCAAGGCT GCGCACCATC ACCACGCGTT TTAGCAGGAT GCGCGCGAT
22621 ATCTTGAAGT GCGAGTTGGG GCGTCCGGCC TCGCGCGCGG AGTTGCGATA CACAGGGTTG
22681 CAGCATCGGA ACACATACAG CCGCGGGTGG TGCAGCTCGG CCGACGCGCT CTGTGCGAG
22741 ATCAGATCCG CGTCCAGGTC TCCCGGTTG CTGAGGCGCA CCGAGGTCAA CTTTGTAGC
22801 TGCTTCCCA AAAAGGGGCG GTGCCACGCG TTTGAGTTGC ACTCGCACCG TAGTGATCT
22861 AAAAGGTGAC CGTGCCCGGT CTGGCGTTA GGATACAGCG CCGTCAATAA AGCCTTGATC
22921 TGCTTAAAAA GCACCTGAGC TTTCGCGCT TCAGAGAAGA ACATGCGCGA AGACTTGCCG
22981 GAAACACTGAT TGGCGGACCA GCGCGCGTGC TGCAGCGAGC ACCTTGGGTC GGTGTGGAG
23041 ATCTGACCCA CATTTGCGCC CACCGGTTCT TTACGATCT TGGCGTGTCT AGACTCGCTC
23101 TTCAGCGCGC GCTGCCGCTT TTGCTCGTGC ACATCCATT CAATCAAGCT CTCCTTATTT
23161 ATCATAAATG TTCGCTGTAG ACACCTTAAG TCGCCTCGA TCTCAGCGCA GCGGTGACAG

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23221 CACAACGCGC AGCCCGTGGG CTCGTGATGC TTGTAGGTCA CCTCTGC AAA GCACTGCAGG  
 23281 TACGCCCTGCA GGAATCGCCC CATCATCGTC ACAAGGTCT TGTGTCTGGT GAAGTGCAGC  
 23341 TGCAACCCCG GGTGCTCCTC GTTCAGCCAG GTCTTGKATA GCGCCGAGGT AGCTTCCACT  
 23401 TGGTCAGGCA GTAGTTTGA A GTTCGCCCTT AGATCGTTAT CCACGTGGTA CTGTGCTCAT  
 23461 AGGCGCGCGC CAGCCTCCAT GCCCTTCTCC CACGCAAGCA CGATCGGCGC AGTACGCGGG  
 23521 TTTATCACCG TAATTTCAC T TCCCGCTTCC CTGGGCTCTT CCTCTTCTCT TTGCGCTCCG  
 23581 ATACACCGCG CCACTGGGTC GTCTTCACT AGCCGCGGCA CTGTGCGCTT ACCTCGCTTTG  
 23641 CCATGCTTGA TTAGCACCG TGGGTGTCTG AAACCCACCA TTTGTAGCGC CACATCTTCT  
 23701 CTTTCTTCTC CGCTGTCCAC GATTACCTTG GGTGATGGCG GCGCTCTGGG CTTGGGAGAA  
 23761 GGGCGCTTCT TTTTCTTCTT GGGCGCAATG GCGCAATCCG CCGCAATCCG CCGTGGCGCG  
 23821 GGGCTGGGTG TGGCGGGCAC CAGCGCGTCT TGTGATAGT CTTCCTCGTC CTGGGACTCG  
 23881 ATACGCCGCC TCATCCGCTT TTTTGGGGG GCGCGGGGAG GCGCGCGCGA CGGGGACGGG  
 23941 GAGCAGACGT CTTCCATGTT TGGGGGACGT GCGCGCGCAC CCGCTCCGCG CTGGGGGGTG  
 24001 GTTTCGCGCT GCTCTCTTC CCGACTGGCC ATTTCTTCT CTATAGGCA GA AAAAGATC  
 24061 ATGGAGTCAG TCGAGAAGAA GGACAGCTA ACCGCCCTCT CTGAGTTGCG CACCACCGCC  
 24121 TCCACCGATG CCGCCAAACG GCCTACCACT TTCCCGCTCG AGGCACCCCC GCTTGAGGAG  
 24181 GAGGAAGTGA TTATCGAGCA GGACCCAGGT TTTGTAAGCG AAGACGAGCA GGACCGCTCA  
 24241 GTACCAACAG AGGATAAAAA GCAAGACCA GACAACGCA AGGCAACGCA GGAACCAAGT  
 24301 GGGCGGGGGG ACGAAAGGCA TGGCGGACTC CTAGATGTGG GAGACGAGCT GTGTGTAGAG  
 24361 CATCTGCGAG GCCAGTGGCC CATTATCTCG GACGCGTTGG AAGAGCGGCG CAGTGTGGCC  
 24421 CTGCGCATAG CCGATGTGAG CCTTGCTTAC GAACGCCACC TATTCTCACC GCGCGTACCC  
 24481 CCGAAAGCGC AAGAAAAAGG CACATGCGAG CCCAACCCCG GCCTCAACTT CTACCCCGTA  
 24541 TTTCGCGTGC CAGAGGTGCT TGGCCACTAT CACATCTTTT TCCAAAACGT CAGATATACC  
 24601 CTATCTGCGC GTGCCAACCG CAGCGGAGCG GCAAGACGAG TGGCTTTGCG GCAGGGCGCT  
 24661 GTCATACCTG ATATCGCCTC GCTCAACGAA GTGCCAAAAA TCTTTGAGGG TCTTGGAGCG  
 24721 GACGAGAGAG CGCGGCAAAA GCTCTGCAAA CAGGAAAAAA AGCTCACTCT AGCTCACTCT  
 24781 GGAGTGTGCG TGGAACTCGA GGTGACAAAC GCGCGCTAG CCGTACTAAA ACGCAGCATC  
 24841 GAGGTCAACC ACTTTGCTTA CCGCGCACTT AACCTACCCC CCAAGGTCTAT GAGCAGATC  
 24901 ATGAGTGAGC TGATCGTGGC CGGTGCGGAG GACGAGCAGC TAGCGCGCTG GCTCTCAAGC  
 24961 CAAACAGAGG AGGGGCTACC CGCAGTTGGC AAACCTAATGA TGGCCGCACT GCTCGTTACC  
 25021 CCGGAGCCTG CCGACTTGA GAGGCGAGCG AAACCTAATGA TGGCCGCACT GCTCGTTACC  
 25081 GTGGAGCTTG AGTGATGCA GCGGTTCTTT GCTGACCCGG AGATGCGAGC CAACTAGAG  
 25141 GAAACATTGC ACTACACCTT TCGACAGGCG TACGTACGCG AGGCGCTGCA GATCTCCAAC  
 25201 GTGGAGCTCT GCAACCTGTT CTCTACTCTT GGAATTTTGC ACGAAAAACCG CCTTGGGCAA  
 25261 AACGTGCTTC ATTCCACGCT CAAGGGCGAG GCGCGCGCGC ACTACGTCCG GAGCTGCGTT  
 25321 TACTTATTTT TATGCTACAC CTGGCAGACG GCCATGGGCG TTTGGCAGCA GTGCTTGGAG  
 25381 GAGTGCAACC TCAAGGAGCT GCAGAAACTG CTAAAGCAAA ACTTGAAGAA CTTATGAGCG  
 25441 GCCTTCAAGC AGCGCTCCGT GCGCGGCGAC CTGGCGGACA TACTTTTCCC CGAACGCTGT  
 25501 CTAAACACCC TGCAACAGGG TCTGTCAGAC TCTACCACTG AAAGCATGTT CAGAACTTT  
 25561 AGGAACCTTA TCCTAGAGCG CTCAGGAATC TTGCGCGCCA CCTGCTGTGC ACTTCTAGC  
 25621 GATCTTGTGC CCAATTAAGTA CCGCGAATGC CCTCGCGCGC TTTGGGCGCA CTGCTACTCT  
 25681 CTGACGTGAG CCAACTACTT TGCTTACCAC TCTGACATAA TGGAAAGCGT GAGCGGTGAC  
 25741 GGTCTACTGG AGTGTCACGT TCGCTGCAAC CATATGCAACC CGCACCGCTC GAGCGTTTGC  
 25801 AATTGCGAGC TGCTTAACGA AAGTCAAAAT ATCGGTACTT TTGAGCTGCA GGGTCCCTCG  
 25861 CACTGACAAA AGTCCGCGGC TCCGGGTTTG AACTCACTC CCGGCTGTGT GAGCTCGGCT  
 25921 TACTTTCGCA AATTGTGACC TGAGGACTAC CAGCCCGACG AGATTATGTT TACGAGAGC  
 25981 CAACCCCGCC GCGCAATGCG GGAGCTTACC GCTCTGCTTA TTACCCAGCA CCACTTCTT  
 26041 GGCCAAATGC AAGCCATCAA CAAAGCCCGC CAGAGTTTTC TGCTACAAA GGGAGCGGGG  
 26101 GTTTACTTGG ACCCCGAGTC CCGCGAGGAG CTCACCCCAA TGTCCCGGCC CCGCCGCGCC  
 26161 TATCAGCAGC AGCCCGCGGC CCTTGCTTCC CAGGATGGCA CCGCAAAAGA AGCTGCACT  
 26221 CGCGCGCCCA CCCACGAGCG AGGAGGAATA CTGGGACAGT CAGGCGAGAG AGGTTTGGAG  
 26281 CAGAGGAGAG GAGGACATGA TGGAAAGCTA GAGAGCGCTA GACGAGGAG CTCTCGAGGT  
 26341 CAGAGGAGGT TCAGACGAAA CACCGTCACT CTGCGTCACT TTCCCTTCGC CCGCGGCCCA  
 26401 GAAATCGGCA ACCGGTTCCA GATGTGCTCA AACCTCCGT CCTCAGAGCG CGCGCGCACT  
 26461 GCGCGTTGCG GCACCCAAAC GTAGATGGGA CACCACTGGA ACAGGCGCGG GATGAGTCAA  
 26521 GCGCCGCGCG CGGTTAGCCG AAGAGCAACA AGACTGTGGG GGCACATCT CTCTGCGCG  
 26581 GCACAAGAAC GCCATAGTGT CTGTCTGCA AGACTGTGGG GGCACATCT CTCTGCGCG

ad5

 FIGURE 21  
 (SHEET 8)

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26641 CCGCTTTCTT CTCTACCATC ACGGCGTGGC CTTCGCCCTG AACATCTCTG ATTACTACGG  
 26701 TCATCTCTAC AGCCCATACT GCACCGGCGG CAGCGCGAGC GGCAGCAACA GCAGCGCGCA  
 26761 CA CAGAAGCA AAGGCGACCG GATAGCAAGA CTCTGACAAA GCCCAAGCAA TCCACAGCGG  
 26821 CGGCAAGCAG AGGAGGAGGA GGGCTGCGTC TGGCGGCCAA CGAACCCGTA CGGACCCGCG  
 26881 AGCTTAGAAA CAGGATTTTT CCCACTCTGT ATGCTATATT TCAACAGAGC AGGGGCGCAAG  
 26941 AACAGAGGCT GAAAATAAAA AACAGGCTCT TGCATCCCTT CACCCGAGCG TGCCCTGTATC  
 27001 ACAAGAAAGCA AGATCAGCTT CGCGCGCAGC TGGAGAGCGC GGAGGGCTCTC TTGAGTAAAT  
 27061 ACTCGCGCTC GACTCTTAAG GACTAGTTTG CGCGCCCTTC TCAAAATTTA GCGCGAAAAC  
 27121 TAGCTCATCT CCAGCGGCCA CACCCGGCGC CAGCACCTGT CGTCAGCGCC ATTATGAGCA  
 27181 AGGAAATATCC CACGCGCTAC ATGTGGAGTT ACCAGCCACA AATGGGACTG GCGGCTGGAG  
 27241 CTGCCCCAAGA CTACTCAACC CGAATAAACT ACATGAGCGC GGGACCCAC ATGATATCCC  
 27301 GGGTCAACGG AATCCGCGCC CACCGAAAAC GAATTCCTCT GGAACAGGCG GCTATTACCA  
 27361 CCACACCTCG TAATAACCTT AATCCCCGTA GTTGGCCCGC TGCCCTGGTG TACCAGGAAA  
 27421 TGTCCGCTCC CACCACTGTG GTACTTCCCA GAGACGCCCA GGCAGAAGTT CAGATGACTA  
 27481 ACTCAGGGGC GCAGCTTGGC GGGCGCTTTC GTCACAGGCT CGCGTCGCCC GGGCAGGGTA  
 27541 TAACTCACCT GACAATCAGA GGGCGAGGTA TTCAGCTCAA CGACGAGTCT GGTAGCTCCT  
 27601 CGCTTGGTCT CCGTCGGGAC GGGACATTTC AGATCGGCGG CGCCGCGCGT CCTTCATTCA  
 27661 CGCTCTGTGA GGCAAATCCTA ACTCTGACGA CTCTGCTCTC TGAGCGCGGT CTGAGGGGCA  
 27721 TTGGAATCTG GCAATTTATT GAGGAGTTTG TGCATCGGCT CTACTTTAAC CCGCTCTCGG  
 27781 GACCTCCCGG CCACTATCCG GATCAATTTA TTCTAACTTT TGAGCGGGTA AGGACTTCGG  
 27841 CGGACGGCTA CGACTGAATG TTAAGTGGAG AGGCAGAGCA ACTCGCGCTG AAAACACTGG  
 27901 TCCACTGTGC CGGCCACAAG TGCTTTGCGG GCGACTCCGG TGAATTTCTG TACTTTGAAT  
 27961 TGCCCGAGGA TCATATCGAG GGGCCGCGCG ACGGCGTCCG GCTTACCGCG CAGGGAGAGC  
 28021 TGCCCGTAGA CTGATTCGCG GAGTTTACCC AGCGTCTGAG GCTAGTTGAG GGGGACAGGG  
 28081 GACCCTGTGT TCTCACTGTG ATTTGCAACT GTCTTAACCT TGGATTACAT CAAGAICTTT  
 28141 GTTGCATCTT CTGTGCTGAG TATAATAAAT ACAGAAATTA AAATATAGTG GGGCTCTAT  
 28201 CGCCATCCCT TAAACGCCAC CGCTTTCACC CGCCCAAGCA AACCAAGGCG AACCTTACCT  
 28261 GGTACTTTTT ACATCTCTCC CTCTGTGATT TACAACAGTT TCAACCAGA CGGAGTGAGT  
 28321 CTACGAGAGA ACCTCTCGGA GCTCAGTAC TCCATCAGAA AAAACACAC CCTCCTTACC  
 28381 TGCCGGGAAC GTACGAGTGC GTCAACGGCC GCTGCACCAC ACCTACCCCG TGACCGTAAA  
 28441 CCAGACTTTT TCCGACAGCA CCTCAATAAC TCTGTTTTACC AGAACAGGAG GTGAGCTTAG  
 28501 AAAACCCCTA GGGTATTAGG CCAAAGGCGC AGCTACTGTG GGGTTTATGA ACAATTCAG  
 28561 CAACTCTAGG GGCATTCTTA ATTCAAGTTT CTCTGAATC CTCTGAATC TTATTCTCTG  
 28621 TCTTGTGATT CTCTTTATTC TTATACTAAC GCTTCTCTGC CTAAGGCTCG CGCGCTGCTG  
 28681 TGTGCACATT TGCAATTTAT GTCACTTTT TAAAGCTGG GGTGCGCAC CAAGATGATT  
 28741 AGGTACATAA TCTAGGTTT ACTCACCTT GCGTCAGCCC ACGGTACAC CCAGAGGGTG  
 28801 GATTTTAAAG AGCCAGCCTG TAATGTTACA TTGCGAGCTG AAGCTAATGA GCAACACTG  
 28861 CTTATAAAAT GCACCAAGA ACATGAAAG CTGCTTATTC GCCACAAAA CAAATTTGCG  
 28921 AAGTATGCTG TTTATGCTAT TTGGCAGCCA GGTGACACTA CAGAGTATAA TTTTACAGTT  
 28981 TTCCAGGGTA AAAGTCATAA AACTTTTATG TATACTTTTC CATTTTATGA TTTGAGCGAC  
 29041 ATTACCATGT ACATGAGCAA ACAGTATAAG TTGTGGCCCC CACAAAAATG TGTGAAAAAC  
 29101 ACTGGCATT TCTGTGCACT TGCTATGCTA ATTACAGTGC TCGCTTTGGT CTGTACCTTA  
 29161 CTCTATATTA AATACAAAAG CAGACGCGAG TTTATTGAGG AAAGAAATG CCGCTTAATT  
 29221 ACTAAGTTAC AAAGCTAATG TCACACTTAA CTGCTTTACT CGCTGTGTC AAAACAAT  
 29281 CAAAAGGTTA GCAATTAAAT TAGAATAGGA TTTAAACCCC CCGGTCAATT CCGTCTCAAT  
 29341 ACCATTCCCC TGAACAATG ACTCTATGTG GGTATGTCT CAGCGCTACA ACCTTGAAGT  
 29401 CAGGCTCTCT GGAATGCAGC ACTGACTTTT GGGCAGCAC TGTCCCGCGG ATTTGTCCCA  
 29461 GTCCAACTAC AGGCAACCCAC ATCTACAGAG ATGACCAACA CAACCAAGCG GCGCGCGCTG  
 29521 ACGGCACTTA CATCTACACC AATACACCC CAAGTTTCTG CTTTGTGCAA TAACTGGAGT  
 29581 AACTTGGGCA TGTGGTGGTT TCTCATGCG CTTATGTTTG TATGCGTTAT TATTATGTTG  
 29641 CTCATCTGCT GCCTAAAGCG CAAAAGCGCC CGACCAACCA CTATAGTCCC CATCATTTTG  
 29701 CTACACCCAA ACAATGATGG AATCCATAGA TTGACGCGAC TGAAACACAT GTATTTCTCT  
 29761 CTATCAGATT GATTAAATGA GACATGATTC CTGAGTGTCT TATATTACTG ACCCTGTGTT  
 29821 CGCTTTTGTG TGGGTGCTCC ACATTGGCTG CGGTTTCTCA CTGGAAGTA GACTGACTTC  
 29881 CAGCCTTCACT AGTCTATTGT CTTTACGGAT TTGTCAACCT CACGCTCATC TGACGCTACA  
 29941 TCACCTGTGT CATCGCCCTT ATCCAGTGC TTGACTGGGT CTGTGTGGCG TGTGTATATC  
 30001 TCAGACACCA TCCCCAGTAC AGGGACAGGA CTATAGCTGA GCTTCTTAGA ATTCTTTAAT

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30061 TATGAAATTT ACTGTGACTT TTCTGCTGAT TATTTCGACC CTATCTGCGT TTGTGTCCCC
30121 GACCTCCCAAG CCTCAAAGAC ATATATCATG CAGATTCACT CGTATATGGA ATATTCCAAAG
30181 TTGCTACAAT GAAAAAAGCG ATCTTTCCGA AGCCTGGTTA TATGCAATCA CTCTCTGTTAT
30241 GGTGTTCTGCG AGTACCATTCT TAGCCCTAGC TATATATTCCC TACCTTGACA TTGGCTGGAA
30301 ACGAATAGAT GCCATGAACC ACCCAACTTT CCCCGCGCCC GCTATGCTTC CACTGCAACA
30361 AGTTGTTGCC GCGGCGCTTG TCCAGCCAA TCAGCCTCGC CCCACTTCTC CCACCCCCAC
30421 TGAATCAGC TACTTTAATC TAACAGGAGG AGATGACTGA CACCTTAGAT TATGAAATGG
30481 ACGGAATTAAT TACAGAGCAG CGCTGTGCTAG AAAGACGCGAG GGCAGCGGCC GAGCAACAGC
30541 GCATGAATCA AGAGCTCCAA GACATGGTTA ACTTGCACCA GTGCAAAAGG GGTATCTTTT
30601 GTCTGTGTAA GCGAGCCAAA GTCACTACG ACAGTAAATC CACCGCAACAC CCGCTTAGCT
30661 ACAAGTTTGG AACCAAGCGT CAGAAATTGG TGGTCAATGG GGGAGAAAGG GGTATATCCA
30721 TAACTCAGCA CTCGGTAGAA ACGGAAGGCT GCATTCACTC ACCTTGTGCA GGAOCTGAGG
30781 ATCTCTGCAC CTTTATTAAG ACCCTGTGCG GTCTCAAGA TCTTATTCCC TTTAATCTAAT
30841 AAAAAAAAT AATAAAGCAT CACTTACTTA AAATCAGTTA GCAAATTTCT GTCCAGTTTA
30901 TTCAGCAGCA CTCTCTTGCC CTCCTCCAG CTCTGGTATT GCAGCTTCTT CTCTGGCTGA
30961 AACTTTCTCC ACAATCTAAA TGAATGTGA GTTTCCTCCT GTTCTGCTCC ATCCGCAACC
31021 ACTATCTTCA TGTGTGTGCA GATGAAGCGC GCAAGACCGT CTGAAGATAC CTCAACCCC
31081 GTGTATCCAT ATGACACGGA AACCGGTCCT CCAACTGTGC CTTTCTCTAT TCCTCCCTTT
31141 GTTCCCCCA ATGGGTTTCA AGAGAGTCCC CTTGGGGTAC TCTCTTTGCG CTTATCCGAA
31201 CCTCTAAGTTA CCTCCAATGG CATGCTTGGC CTCAAAATGG GCAACGGGCT CTTCTGTGAG
31261 GAGGCGCGCA ACCTTACCTC CCAAAATGTA ACCACTGTGA GCCCACTCTT CAAAAAATCT
31321 AAGTCAAACTA TAAACCTGGA AATATCTGCA CCCCCTCAGA TTACTCTAGA AGCCCTAACT
31381 GTGCTGTCGG CGCACCTCTT AATGTTGCGG GGCACACAC TCACCATGCA ATCAAGGCC
31441 CGCTTAACCG TGCACGACTC CAAACTTAGC ATTGCGACCC AAGGACCCCT CACAGTGTCT
31501 GAAAGAAAGC TAGCCCTGCA AACATCAGCG CCCCCTACCA CCACCGATAG CAGTACCCCT
31561 ACTATCACTG CTTCAACCCC TCTAACTACT GCCACTGGTA CTTGTGGCAT AGCTTGTGAA
31621 GAGCCCATTT ATACACAAAA TGGAAAACTA GGACTAAAGT ACGGGGCTCC TTGTCATGTA
31681 ACAGACGACC TAAACACTTT GACCGTAGCA ACTGGTCCAG GTGTGACTAT TAATAATACT
31741 TCCTTGCAAA CTAAGTTTAC TGGAGCCTTG GGTTTTGATT CACAAGCGAA TATGCAACTT
31801 AATGTAGCAG GAGGACTAAG GATTGAITCT CAAAACAGAC GCCTTTACTT TGAITTAGT
31861 TATCCGTTTG ATGTCTAAAA CCAACTAAAT CTAAGACTAG GACAGGGCCC TCTTTTATA
31921 AACTCAGCCC ACAACTTGGA TATTAACTAC ACAAAGGCC TTTACTTGTG TACAGCTTCA
31981 AACAATTTCCA AAAAGCTTGA GGTAAACCTA AGCACTGCCA AGGGGTTGAT GTTTGAGCTG
32041 ACAGCCATAG CCATTAATCG AGGAGATGGG CTTGAATTTG GTTCACCTTA TGCACAAAC
32101 ACAAATCCCC TCAAAACAAA AATTGGCCAT GGCTAGAAAT TTGATTCAAA CAAAGCTATG
32161 GTTCTTAAAC TAGGAACCTG CCTTAGTTTT GACAGCAGC GTGCCATTAC AGTAGAAAC
32221 AAAAAATAAG ATAAGCTAAC TTTGTGGACC ACACGACAT CATCTCCTTA CTCTGAGACT
32281 AATCGAGAGA AAGATGTCAA ACTCACTTTG GTCTTAAACA AATGTGGCAG TCAAAATCTT
32341 GCTACAGTTT CAGTTTTGGC TGTTAAAGGC AGTTTGGCTC CAATATCTGG AACAGTTCAA
32401 AGTGCTCATC TTATTATAAG ATTTGACGAA AATGGAAGTG TACTAAACAA TTCTTCTCTG
32461 ACCCGAGAAAT ATTGGAACIT TAGAAATGGA GATCTTACTG AAGGCACAGA GTATACAAAC
32521 CGCTGTTGAT TTATGCTCAA CCTATCAGCT TATCCAAAA CTACCGGTAA AACTGCGAAA
32581 AGTAAACATG TCAGTCAAGT TTACTTAAAC GGAGACAAAA CTPAACCTGT AACACTAACC
32641 ATTAACATAA ACGGTACACA GGAACAGGGA GACACAACCT CAAGTGCATA CTCTATGTCA
32701 TTTTCTGCG ACTGGTCTGG CCACAACTAC ATTAATGAAA TATTTGCAC ATCTCTTATC
32761 ACTTTTTCAT ACATTGCCCA AGAATAAAGA ATCGTITGTG TTATGTTTCA ACGTGTITAT
32821 TTTTCAATTG CAGAAAAATT CAACTCATTT TTCAITTCAGT AGTATAGCCC CACCAACACA
32881 TAGCTTATAC AGATCAACCGT ACCTTAATCA AACTCACAGA ACCCTAGTAT TCAACCTGCC
32941 ACCTCCCTCC CAACACACAG AGTACACAGT CCTTCTCCTC CCGCTGGTCC TAAAGAGCAT
33001 CATATCATGG GTAACAGACA TATCTTAGG TGTATATTC CACACGGTTT CCTGTGAGAG
33061 CACAGCCTCA TCAGTGAAT TAAATAACT CCCGCGCAG CTCACTTAAG TCAATCTCGT
33121 GTCCAGCTGC TGAGCCACAG GCTGCTGTCC AACTTCCGGT TGCTTAAOAG GCGCGCAAGG
33181 AAGAGTCCAC GCCTACATGG GGGTAGAGTC ATAATCGTAG ATCAGATAGG GCGCGGTGGT
33241 CTGACGACAG CGCGAATAAA ACTGCTGCGC CCGCGCTCC GTCTCGAGG ATATCAACTAT
33301 GCGAGTGGTC TCCTCAGCGA TGATTGCGAC CGCGCGCAGC ATAGGCGGCT TTGTCTCGCG
33361 GGCACAGCAG CGCACCTCGA TCTCACTTAA ATCAGCAGAG TAACTCGAGC ACAGCACCAC
33421 AATATTGTTT AAAATCCCAC AGTGCAGGCG GCTGTATCCA AAGCTCATGG CCGGAGCCAC

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33481 AGAAGCCAGC TGCCATCAT ACCACAAGCG CAGGTAGATT AAGTGGCGAC CCTCTATAAA
33541 CACGCTGGAC ATAAACATTA CCTCTTTTGG CATGTTGTAA TTCACCACTC CCGGTATACA
33601 TATAAACCTC TGATTAAACA TGGCGCCATC CACCAACATC CTAACACGAGC TGGCCAAAAC
33661 CTGCCCGCGC GCTATACACT GCAGGGAACC GGGACTGGAA CAATGACAGT GAGAGAGCCCA
33721 GGACTCGTAA CCATGGATCA TCATGCTCGT CATGATATCA ATGTTGGCAC AACACAGGCA
33781 CACGTGCATA CACTTCTCTA GGATTACAAAG CTCCTCCCGC GTTAGAACCA TATCCAGGCG
33841 AACAAACCAT TCTGAATCA GCGTAAATCC CACACTGCAG GGAAGACTCT GCACTGAATC
33901 CACGTTGTGC ATTGTCAAAG TGTTACATTC GGGCAGCAGC GGATGATCTC CCAATATGTT
33961 AGCGCGGGTT TCTGTCTCAA AAGGAGGTAG ACGATCCCTA ACGCCGAGCG TAGTCAATAT
34021 CAACCGAGAT CGTGTGTGCT GTAGTGTCTA GCCAAATGGA ACGCCGAGCG TAGTCAATAT
34081 TCCCTGAAGCA AAACACAGGT GCGGCGGTGAC AAGACAGATCT CGCTCTCCGG TCTCGCCGCT
34141 TAGATCGCTC TGTGTAGTAG TTGTAGTATA TCCACTCTCT CAAAGCATCC AGGCGCCCCC
34201 TGGCTTCGGG TTCTATGTAA ACTCCTTCAT GCGCGCGTGC CCTGATACCA TCCACCAAGC
34261 CAGAATAAGC CACACCCAGC CAACCTACAC ATTCGTTCTG CGAGTCAACG ACGGAGAGAG
34321 CGGGAAGAGC TGAAGAAGC ATGTTTTTTT TTTTATTCCA AAAGATTATC CAAACCTCA
34381 AAATGAAGAT CTATTAAAGT AACCGCTTCC CTTCCGGTGG CGTGGTCAAA CTCTACAGCC
34441 AAAGAACAGA TAATGGCATT TGTAAAGATG TGCACAATGG CTTCACAAAG GCACACGGCC
34501 CTCACGTCCA AGTGGACGTA AAGGCTAAAC CCTTCAGGGT GAATCTCCTC TATAACATT
34561 CCAGCACCTT CAACCATGCC CAAATAATTC TCATCTCGCC ACCTTCTCAA TATATCTCTA
34621 AGCAAAATCCC GAATATTAAAG TCCGGCCATT GTAAAAATCT GCTCCAGAGC GCGCTCCACC
34681 TTCAGCCTCA AGCAGCGAAT CATGATTGCA AAAAATTCAGG TTCTCTACAG ACCTGTATAA
34741 GATTCAAAAG CGGAACATTA ACAAATAATC CGCGATCCGG TAGGTCCCTT CGCAGGGCCA
34801 GCTGAACATA ATCGTGCAGG TCTGCACGGA CAGCGCGCGC CACTTCCCGC CAGGAACTC
34861 TGACAAAAGA ACCCACTGT ATTATGACAC GCATACTCGG AGCTATGCTA ACCGCGTAG
34921 CCGGATGTA AGCTTTGTG CATGGCGCGC GATATAAAT GCACGGTGTG GCTCAAAAAA
34981 TCAGGCAAGC CCTCGCGCAA AAAAGAAAGC ACATCGTAGT CATGCTCATG CAGATAAAGG
35041 CAGGTAAGCT CGGGAACCA CACAGAAAAA GACACCAATT TTCTCTCAA CATGCTGCG
35101 GGTTCCTGCA TAAACACAAA ATAAATAAAC AAAAAACAT TTAACATTA GAAGCTGTCT
35161 TTACAAACAGG AAAAAACCC CTTATAAGCA TAAGACGAGC TACGGCCATG CCGCGGTGAC
35221 CGTAAAAAAA CTGCTCAGCG TGATTAAAAA GCACCAACGA CAGCTCTCGT GCTATGTCCG
35281 GAGTCATAAT GTAAGACTCG GTAACACAT CAGGTTGATT CATCGTCAAG TGCTAAAAAG
35341 CACCGGAAAT AGCCCGGGGG AATACATACC CGCAGCGGTA GAGACAACTA TACAGCCCCC
35401 ATAGGAGGTA TAAACAAATT AATAGGAGAG AAAAAACAT AACAACCTGA AAACCCCTCC
35461 TGCTAGGCCA AATAGCACCC CTCCCGCTCC AGACACCAAT ACNCGCTTCC AAGAGCTTCC
35521 CCTAACAGTC AGCCTTACCA GTAAAAAAGA AACCTATTA AAAAAACCC ACTCGACAGC
35581 GCACCAAGCT AATCAGTCAC AGTGTAAAAA AGGCGCAAGT GCAGAGCGAG TATATATAGG
35641 ACTAAAAAAT GAGCTAACGG TTAAGGTCCA CAAAAAACCC CCAGAAAAAC CACGCGAAGC
35701 CTACGCCGAG AAACGAAGC CAAAAACCCC ACAACTTCTT CAAATCTGCA CTTCGTTTTT
35761 CCCAGCTTAC GTAACCTCCC ATTTTAAAGAA AACTACAATT CCAACACTAT ACAATTTACT
35821 CGCCCTTAAA ACCTACGTCA CCGCGCCCGT TCCCAAGCCC CGCGCCACGT CACAAATCTC
35881 ACCCCCTCAT TATCATATTG GCTTCAATCC AAAATAAGGT ATATTATTGA TGATG

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FIGURE 21  
(SHEET 11)

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LOCUS KD1 33592 bp DNA SYN 26-APR-1999

DEFINITION KD1

ACCESSION KD1

KEYWORDS

SOURCE Unknown.

ORGANISM Unknown

REFERENCE 1 (bases 1 to 33592)

AUTHORS Self

JOURNAL Unpublished.

FEATURES

CDS

Location/Qualifiers

1..33592

/gene="KD1"

/product="KD1"

BASE COUNT 7744 a 9470 c 9285 g 7093 t

ORIGIN

1 CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG GGGGTGGAGT

61 TTGTGACGTG CGCGCGGGCG TGGGAACGGG CGCGGTGACG TAGTAGTGTG CGCGAAGTGT

121 GATGTTGCAA GTGTGGCGGA ACACATGTAA CGACCGGATG TGGCAAAAGT GACGTTTGTG

181 GTGTGCGCGG GTGTACACAG GAAGTGACAA TTTTCGCGCG GTTTTAGGGG GATGTTGTAG

241 TAAATTTTGG CGTAAACGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA

301 AGTGAATCT GAATAATTTT GTGTTACTCA TAGCGGTAA TATTGTCTA GGGCGCGGG

361 GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT CTCAGGTGTT TTCGCGGTTT

421 CGGTCGAAAG TTGGCGTTTT ATTATTATAG TCAGCTGACG TCAGCTGACG AGTTTCTCTC TCCGAGCCG

481 TGAGTCTCTC AAGAGGCCAC TCTTGAGTGC CAGCGAGTAG AGTTTCTCTC TCCGAGCCG

541 TCCGACACCG GAGCTGAAAA TGAGACATGA GGTACTGGCT GATAATCTTC CACTCTCTAG

601 CCAATTGAA CCACCTACCC TTCACGAACT GTATGATTTA GACGTGACGG CCCCGRAGA

661 TCCCAACGAG GAGGCGTTTT CGCAGATTTT TCCCGACTCT GTAATGTGCG CGGTGCAGGA

721 AGGGATTGAC TTACTCACTT TTCCGCGCGC GCCCGGTTCT CCGGAGCCGC CTCACCTCTT

781 CGGTCACGCC GAGCAGCCGG AGCAGAGAGC CTTGGGTCCG GTTTGCCACG AGGCTGGCTT

841 TCACCCCACT GACGACGAGG ATGAAGAGGG TGAGGAGTTT GTGTAGATT ATGTGGAGCA

901 CCCCAGGAG GTTTGCAGGT CTTGTCAAT TCACCGAGG AATACGGGGG ACCCAGATAT

961 TATGTGTTCC CTTTGCTATA TGAGGACCTG TGCGATGTTT GTCTACAGTA AGTGAATAAT

1021 ATGGCGCAGT GGTGATAGAG TGGTGGGTTT GGTGTGGTAA TTTTTTTTTT AATTTTTTACA

1081 GTTTTGTGGT TTAAGAATTT TTGTATTGTG ATTTTTTTAA AAGGTCCTGT GTCTGAACCT

1141 GAGCCTGAGC CCGAGCCAGA ACCGAGGACT GCAGACCTA CCGCCGCTCC TAAAATGGCG

1201 CTTGTATATC TGAGACGCCC GACATCACTT GTGTCTAGAG AATGCAATAG TAGTACGGAT

1261 AGCTGTGACT CCGGTCTCTC TAAACACACT CTGAGATAC ACCCGTGGT CCGCTGTGCG

1321 CCATTAAAC CAGTTCCCGT GAGAGTTGGT GGGCGTCCGC AGGCTGTGGA ATGTATCGAG

1381 GACTTGCTTA ACGAGCCTGG GCACCTTTTG GACTTGAGCT GTAACCGCCC CAGGCCATAA

1441 GGTGTAAACC TGTGATTGCG TGTGTGGTAT AGCCTTTTGT TTGCTGAATG ACTTGAATGA

1501 AGTTTAATTA AGGGTGAGAT AATGTTTAAC TTGCATGGCG TGTAAATGTT GGGCGGGCTT

1561 AAAGGGTATA TAAATGCGCG TTGGCTAATC TTGGTTACAT CTGACCTCAT GAGAGCTTGG

1621 GAGTGTGTTG AAGATTTTTC TGCTGTGCGT AACTTGTGCG AACAGAGCTC TACAGTATCC

1681 TCTTGGTTTT GGAGGTTTTT GTGGGCGCTA TCCGAGCAAA AGTTAGTCTC CAGAATTAAG

1741 GAGGATTACA AGTGGGAATT TGAAGAGCTT TTGAATCTCT GTGGTGAAGT GTTTGATTTT

1801 TTGAATCTGG GTCAACAGGC GCTTTTCCAA GAGAAGGTCA TCAAGACTTT GGATTTTTTC

1861 ACACCGGGGC GCGCTCGCGC TGCTGTGCTT TTTTGAAGT TTATAAAGGA TAAATGGAGC

1921 GAGAATAACC ATCTGAGCGG GGGGTACCTG CTGAGTTTTT TGGCATGATA TCTGTGGAGA

1981 CGGGTTGTGA GACACAGAAA TCGCCTGCTA CTGTTGTCTT CCGTCCGCCC GGCATTAATA

2041 CCGACGGAGG AGCAGCAGCA GCAGCAGGAG GAAGCAGGCG GCGCGCGGCA GGAGCAGAGC

2101 CCATGGAACC CGAGAGCCCG CCGGACCTCT CCGGAATGAA TGTGTGACAG GTGGCTGAAC

2161 TGTATCCAGA ACTGAGACGC ATTTTACAAA TTACAGAGGA TGGCGAGGGG CTTAAGGGGG

2221 TAAAGAGGGA CGCGGGGGCT TGTGAGGCTA CAGAGGAGGC TAGGAATCTA GCTTTTAGCT

2281 TAATAGCAGC ACACCGTCTT GAGTGATTA CTTTTCACCA GATCAAGGAT AATTGCGCTA

2341 ATGAGCTTGA TCTGCTGGGG CAGAAGTATT CCAATAGAGC GCTGACCACT TACTGCTGCG

2401 AGCCAGGGGA TGATTTTGAG GAGGCTATTA GGGTATATGC AAAGGTGSCA CTTAGGCCAG

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FIGURE 22  
(SHEET 1)

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2461 ATTGCAAGTA CAAGATCAGC AAACCTGTAA ATATCAGGAA TTGTTGCTAC ATTTCTGGGA  
 2521 ACGGGGCGGA GGTGGAGATA GATACGGAGG ATAGGGTGCC CTTTAGATGT AGCATGATTA  
 2581 ATATGTGTGCC GGGGGTGCTT GGCATGGACG GGGTGTTATG TATGAAATGA GTTGTACTGT  
 2641 GCGCCAAATT TAGCGGTACG GTTTTCTCGG CCAATACCAA CCTTATCTCTA CACGGGTGTA  
 2701 GCTTTCTATT GTTTAAACAT ACCTGTGTGG AAGCCTGGAG CGATGTGAAG GTTCGGGGCT  
 2761 GTGCTCTTTA CTGCTGCTGG AAGGGGGTGG TGTGTGCGCC CAAAAGCAGG GCTTCAATTA  
 2821 AGAATGCGCT CTTTGAAGGG TGTACTTTGG GTATCTGTCT TGAGGGTAACT TCGAGGGTGC  
 2881 GCCACAATGT GGCCTCCGAC TGTGTTGTCT TCATGCTAGT GAAAGACGTG GCTGTGATTA  
 2941 AGCATAAACAT GGTATGTGGC AACTGCGAGG ACAGGGCCTC TCAATGCTGT ACCTGCTGCG  
 3001 ACGGCAACTG TCACCTGTCTG AAGACCAATC ACGTAGCCAG CCACTCTGCG AAGGCTTGCG  
 3061 GCGTTTGTGA GCATAACATA CTGACCCTGT GTTCTTTGCA TTGGGTAACT AGGAGGGGGG  
 3121 TGTTCCTACC TTACCAATGC AATTGTAGTC ACATTAAGAT ATTGCTTAGG CCGGAGGCA  
 3181 TGTCCAAGGT GAACCTGAAC GGGGTGTTTG ACATGACCAT GAAGATCTGG AAGGTGCTGA  
 3241 GGTACGATGA GACCCGACAC AGGTGACAGC CCGTGGAGTG TGCGGTTAA CATATTAGGA  
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 3361 GCACCCGCGC TGAGTTTGGC TCTAGCATG AAGATACAGA TTGAGGTACT GAAATGTGTG  
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 3541 CATATTGAGT AACCGCGCATC CCCCCATGGG CCGGGGTGCG TCAGAAATGT ATGGGCTCCA  
 3601 GCAATTGATG TCGCCCCGTC CTGCCGCGAA ACTCTACTAC CTGACCGTAT GAGACCGTGT  
 3661 CTGGAAACGC GTTGGAGACT CGACGCTCCG CGCGCGCTTC AGCCGTGCA GCCACCGCCC  
 3721 CGGGGATGTG GACTGACTTT GCTTCTCTGA GCGCGATTGC AAGCAGTCTG ACTTCCCGTT  
 3781 CATCGCGCCG CGATGACAAG TTGACGGCTC TTTTGGCACA ATTGGAATCT TTGACCGGGT  
 3841 AACTATAGCT GCTTCTCAG CAGCTGTGG ATCTGCGCCA GCAGGTTTCT GCCCTGAAGG  
 3901 CTCTCTCCCC TCCCAATGCG GTTTAAACAA TAAATAAAAC ACCAGACTGT TTTTGGATTT  
 3961 GGATCAAGCA AGTGTCTTGC TGTCTTATT TAGGGGTTTT GCGCGCGCTG TAGGCGCGGG  
 4021 ACCAGCGGTC TCGGTGTTG AGGGTCTCTG GTATTTTTTC CAGGACGTGG TAAAGGTGAC  
 4081 TCTGATGTTT CAGATACATG GGCATAAGCC CGTCTCTGGT GTGGAGTACT CACCCTGCA  
 4141 GAGCTTACAT CTGCGGGGTG GTGTTGTAGA TGATCCAATC GTAGCAGGAG CCGTGGGCGT  
 4201 GGTGCTTAAA AATGCTTTTC AGTAGCAAGC TGATGGCCAG GGGCAGCGCC TTGGTGTAA  
 4261 TGTTTACAAA CCGGTTAAGC TGGGATGGGT GCATACGTGG GGATATGAGA TGCACTTGG  
 4321 ACTGTATTTT TAGGTTGCTT ATGTTCCCAAG CATATCCCT CCGGGGATAT ATGTTGTGCA  
 4381 GAACCACCAAG CACAGTGTAT CCGGTGCACT TGGGAAATTT GTCATGTAGC TTAGAAGGAA  
 4441 ATGCGTGGAA GAACCTGGAG ACGCCCTTGT GACCTCCAAG ATTTTCCATG CATTCGTCCA  
 4501 TAATGATGGC AATGGGCCCA CGGGCGCGCG CCGGGCGGAA GATATTTCTG GATCACTAA  
 4561 CGTCAATGTT GTGTTCCAGG ATGAGATCGT CATAGGCCAT TTTTACAAAG CGCGGGCGGA  
 4621 GGGTGCCAGA CTGCGGTATA ATGTTTCCAT CCGGGCCAGG GCGGTAGTTA CCGTACAGA  
 4681 TTGTGATTTT CCACGCTTTG AGTTCAGATG GGGGGATCAT GTCTACTCTG TGAGCGATGA  
 4741 AGAAACCGGT TTCCGGGTTA GGGGAGATCA GCTGGGAAGA AAGCAGGTCT CTGAGCAGCT  
 4801 GCGACTTACC GCACGCGGTG GGGCCGTAAA TCACACCTAT TACCGGGTGC AACTGTGATG  
 4861 TAAGAGAGCT CGAGCTGCGG TCATCCCTGA GCAGGGGGCG CACTTCTGTTA AGCATGTCCC  
 4921 TGACTCGCAT GTTTTCCCTG ACCAAATCCG CCAGAAGGCG CTGCGCGGCC AGCGATAGCA  
 4981 GTTCTGTCAA GGAAGCAAG TTTTTCACG GTTTGAGACG GTTCGCGGTA GGTATGCTTT  
 5041 TCGAGGTTTG ACCAAGCAGT TCCAGGCGGT CCCACAGCTC GGTCACCTGC TCTAGGCACT  
 5101 CTGATCCGAC CATATCTCTC CGTTTCCGCG GTTGGGCGCG CTTCCTGTG ACGGCAGTAG  
 5161 TCGGTGCTCG TCCAGACGGG CCAGGGTCAAT GTCTTCCAC GGGCGCAGGG TCCTCTCGAG  
 5221 CGTAGTCTGG GTACCGGTGA AGGGGTGCGC TCCGGGCTCG CGGCTGGCCA GGGTGGCTT  
 5281 GAGGCTGCTC CTGCTGGTGC TGAAGCCTG CCGGTCTTGG CCGGTCTTGG CCGCCAGGTA  
 5341 GCATTTGACC ATGTTGTGAT AGTCCAGCCC CTCCGCGCGG TGCGCTTTGG CCGCAGCTT  
 5401 GCGCTTGGAG GAGGCGCGCG ACAGGGGCA GTGACAGCTT TTGAGGCTGT TTGAGCTTGG  
 5461 CGCGAGAAAT ACCGATTCCG GGGAGTAGGC ATCCGCGCGC CAGGCGCCCG AGACGCTCTC  
 5521 GCATTCCACG AGCCAGGTGA GCTCTGCGCG TTCCGGGTGA AAAACCAAGT TTTCCCAATG  
 5581 CTTTTGTAGT GGTTCCTTAC CTCTGTTTTC CATGAGCGGG TGTCACAGCT CGGTGACGAA  
 5641 AAGGCTGTTC GTGTCCCGGT ATACAGACTT TGAGAGCGCT TCCTCTGAGC GTGTCCGCG  
 5701 CTCTCTCTCG TATAGAAACT CGGACCACTC TGAGACAAAG GCTCGCGCTC AGGCGAGCAC  
 5761 GAAGGAGCCT AAGTGGGAGG GGTAGCGGTC GTTGTCAACT AGGGGGTCCA CTGCTCCAG  
 5821 GGTGTGAAGA CACATGTGCG CCTCTCGCG ATCAAGGAAG GTGATGTGTT TGTAGGTGTA

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FIGURE 22  
(SHEET 2)

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5881 GGCCACGTGA CCGGGTGTTC CTGAAGGGGG GCTATAAAAG GGGGTGGGG GCGGTCGTC  
 5941 CTCACCTCTCT TCCGCATCGC TGCTCGGAG GGCCAGCTGT TGGGGTGAAT ACTCCCTCTG  
 6001 AAAAGCGGGG GTAGACTCTG CGCTAAGATT GTCAAGTTTCC AAAACCGAGG AGGATTGTAT  
 6061 ATTACCATGTT TCCGCGGTGA TGCTTTTAGG GTGGGCGCGA TCCATCTGGT CAGAAAGAC  
 6121 AATCTTTTTT TGTCCAAGCT TGGTGGCAAA GCACCCGTAG AGGGGGTTGG ACAGCAACTT  
 6181 GGGCATGTGAG CGCAGGGTTT GGTTTTTTGT CGCATCGGGG CGCTCCTTTG CCGCATGTTT  
 6241 TAGCTGTGAGC TATTGCGGGC CAACGCACGC CCATTCTGGG AAGACGGTGT TGGCTGTCTC  
 6301 GGGCACCAGG TGACACGGCC AACCGGGTT GTGCAAGGTT ACAAGGTCAC CGCTGGTGGC  
 6361 TACCTCTCCG CGTAGGCGCT CGTTGGTCCA GCAGAGGCGG CCGCCCTTGC GCGAGCAGAA  
 6421 TGGCGGTAGG GGGTCTAGCT GCGTCTCGTC CGGGGGTCT GCGTCCACG TAAAGACCCC  
 6481 GCGCAGACGC CGCGCTCGA AGTAGTCTAT CTTGCTATCT TGCAAGTCT TGCACTGCTG  
 6541 CCAATGCGCGG GCGGCAAGCG CGCGCTCGTA TGGGTGTGAT GGGGGACCCC ATGGCATGGG  
 6601 GTGGGTGAGC GCGGAGGCGT ACATGCGCGA AATGTCGTAA ACGTAGAGGG GCTCTCTGAG  
 6661 TATTTCGAAGA TATGTAGGGT AGCATCTTCC ACCGCGGATG CTGGCGCGCA GGTATGCTGA  
 6721 TGTGCTGTGC GAGGAGCGA GGAGGTCGGG ACCGAGGTTG CTACGGGCGG GCTGCTCTGC  
 6781 TCGGAAGACT ATCTGCTGTA AGATGGCATG TGAGTTGGAT GATATGGTTG GACGCTGGAA  
 6841 GACGTTGAAG CTGGCGTCTG TGAGACCTAC CGCGTACCG ACGAAGGAGG CGTAGGAGTC  
 6901 GCGCAGCTTG TTGACAGCT CGGCGGTGAC CTGCACGTCT AGGGCGCATG AGTCCAGGGT  
 6961 TCTCTTGATG ATGTCTACT TATCTGTGCC CTTTTTTTTT CACAGCTCGC GGTGTAGGAC  
 7021 AAATCTCTTC GCGTCTTTCC AGTACTCTTG GATCGGAAAC CCGTCGGCTC CCGAAGCGTA  
 7081 AGAGCTAGAG ATGTAGNACT GGTGACGGC GTGTAGGCG CAGCATCCCT TTCTCAAGG  
 7141 TAGCGCGTAT GCGTCCGCGG CCTTCCGGAG CGAGGTGTGG GTGAGCGCAA AGGTGTCCCT  
 7201 GACCATGACT TTAGGTAAT GGTATTTGAA GTCACTGTGC TGCACTCCG CCGTCTCCCA  
 7261 GAGCAAAAGG TCGGTGCGCT TTTTGAAGCG CGGATTTGGC AGGGCAGGAG TGACATCTGT  
 7321 GAAGAGTATC TTTCCGCGCG GAGGCATAAG GTTGCCTGTG ATGCGGAAGG GTCCCGGCAC  
 7381 CTGCGAATCG TTGTTAATTA CCTGGGAGGAG GAGCACGATC TGCTCAAGAG GTTGTATGTT  
 7441 GTGGCCCAAC ATGTAAAGTT CCAAGAAGCG CGGAGTGCCT TTGATGGAAG GCAATTTTTT  
 7501 AAGTTCCTCG TAGGTGAGCT CTTCAGGGGA GCTGAGCCCG TGCTCTGAAA GCGCCGAGTC  
 7561 TGCAAGATGA GGGTGTGAAG CGACGAATGA GCTCCACAGG TCACGGGCCA TTAGCATTTG  
 7621 CAGGTGCTCG CGAAAGGTCC TAAACTGGCG ACCTATGGCC ATTTTTTCTG GGGTGAATGA  
 7681 GTAGAAGGTA AGCGGGTCTT GTTCCGAGCG GTCCCATCCA AGGTTCGCGG CTAGGTCTCG  
 7741 CGCGGCAGTC ACTAGAGGCT CATCTCGGCC GAACTTCATG ACCAGCATGA AGGGCACAGG  
 7801 CTGCTTCCCA AAGGCCCCCA TCCAAGTATA GGTCTCTACA TCGTAGGTGA CAAAGAGACG  
 7861 CTGCGTGTGA GATGTGAGC CGATCGGGAA GAACTGGATC TCCCGCCCACT AATTGGAGGA  
 7921 GTGGCTATTG ATGTGGTGA AGTAGAAGTC CTTGCGACGG GCCCAACACT GGTGCTGGCT  
 7981 TTTTGTAAAA CGTGCAGAT ACTGGCAGCG GTGCACGGCG TGTAATCTCT GCACAGGTTT  
 8041 GACCTGAGGA CGCGCACAA GGAAGCAGAG TGGGAATTG AGCCCTCTCG CTGGCGGGTT  
 8101 TGTGTTGGTG TCTTCTACTT CGGCTGCTTG TCTTGTGACC TCTGGCTGTG CTAGGGGAGT  
 8161 TAGGTTGATG CGGACACCA CGCGCGCGCA GCCCAAGTC CAGATGTCCG CGCGCGGGG  
 8221 TGGAGCTTTG ATGACAACAT CGCGCAGATG GGAGCTGTCC ATGGTCTGCA GCTCCGCGG  
 8281 CGTCAAGTCA GCGCGGAGCT CCGTCAAGTT TACTCGCAT AGACGGGTGA GGGCGCGGG  
 8341 TAGATCCGAT TGATACCTAA TTTTCAAGGG CTGTTTGGT GCGGCTCGA GGGCTGTGCA  
 8401 GAGGCGCGAT CCGCGCGGCG CGACTACGAT ACCGCGCGCG GGGCGGTGGG CCGCGGGGT  
 8461 TGCTTTGATG GATGCATCTA AAAGCGGTGA CGCGGGCGAG CCGCGGGAGG TAGGGGGGCG  
 8521 TCGGAGACCG CGGAGGAGG GGGCAGGGCG ACGTGCGGCG CCGCGCGGGG CAGAGCTTGG  
 8581 TGCTGCGCGC GTAGGTTGCT GGGCAACGCG ACGACGCGCG GTTGTATCTC CTGATTTCTG  
 8641 CGCTCTCGCG TGAAGACGAC GGGCGCGGTG AGCTTGAGCT CCGTCAAGAT TGACAGAGTA  
 8701 TCAATTTCCG TGTCGTTGAC GGGCGGCTGG CGCAAAATCT CCGTCAAGAT TCGTCAAGAT  
 8761 TCTTGATAGG GATCTCGGC CATGAACCTC TGATCTCTCT CCGTCTGAG ATCTCCGGGT  
 8821 CCGGCTGCTT CCACGGTGGC GGGCAGGTCG TTGGAATGCG GGGCATAGG CTGCGAGAG  
 8881 GCGTGTGAGC CTCCCTCGTT CCAGACGCGG CTGTAGACCA CGCCCGCTTC GGTGCGGG  
 8941 GCGCGCATGA CCACCTGGCG GAGATTGAGC TCCACGTGCC GGGCGAAGAC GGGTATGTT  
 9001 CGCAGCGGCT GAAAGAGGTA GTTGAAGGTT GTGGCGGTTG GTGCGGCTG GTTCTGCGAC  
 9061 ATAAACCAAGC GTGCSAAGCT GGAATTCGTT ATATCCCAAG AGGCTCAAG GCGCTCCATG  
 9121 TCTGCTGAGA AGTCCACGGC GAAGTTGAAA AACTGGGAGT TGCGCGCGGA CAGGTTAAG  
 9181 TCTCTCTCCA GAAGACGGAT GAGCTCGGGC ACAATGTCCG GCACCTCGCG CTCAAAGGCT  
 9241 ACAGGGGCGT CTTCTCTCTC TTCAATCTCC TTTTCCATA GGGCGCTCCC TTCTCTCTCT

9301 TCTGGCGGCG GTGGGGGAGG GGGGACACGG CGGCGACGAC GGGCGACCGG GAGGCGGTGG  
 9361 ACAAGAGCGCT CGATCATCTC CCCGCGGGCG CGGCGCATGG TCTCGGTGAC GGGCGGGCGG  
 9421 TTCTTCGCGGG GGGCGAGTTG GAAGACGCGG CCGGTGATG CCGGTGATG GGTGTGGCGG  
 9481 GGGTGTCCATC CGGCGAGGGA TACGGCGCTA ACGATGTCAT TCAACAATTG TTGTGTAGGT  
 9541 ACTCCGCGCGC CGAGGGACCT GAGCGAGTCC GCATCGACCG GATCGGAAAA CCTCTCGAGA  
 9601 AAGGCGTCTA ACCAGTCACA GTCCGAAGGT AGGCTGAGCA CCGTGGCGGG CGGACGCGGG  
 9661 CGGCGGTGGG GGTGTGTTCT GGGCGAGGTG CTGCTGATGA TGTAAATATA GTAGGCGGTC  
 9721 TTGAGACGGC GGTGTGGTGA CAGAAGCACC ATGTCTCTGG GTCCGGCGGT CTGAATGGCG  
 9781 AGGCGGTGGG CCATGCCCCA GGCTTCGTTT TGACATCGCG GCAGGTCTTT GTAGTAGTCT  
 9841 TGACATGAGCC TTTCTACCGG CACTTCTTCT TCTCTCTCT CTGTCTCTCG ATCTCTTGA  
 9901 TCTATCGCTG CGGCGGGCGG GAGGTTTGGC GGTAGGTGGC GCCCTCTGCG TCCCATGTCT  
 9961 GTGACCCCGA AGCCCCCTCAT CGGCTGAAGC AGGCTAGGT CGGCGACAA CCGCTCGGCT  
 10021 AATATGGGCT GCTGCAACCT CGTGAGGGTA GACTGGAAGT CATCCATGTC CACAAGCGG  
 10081 TGGTATGCGG CCGTGTGAT GGTGTAAGTG CAGTTGGCCA TAACGGACCA GTTAAAGGTC  
 10141 TGGTGACCCG GCTCGAGAG CCGGTGTGAC CTGAGACCGG AGTAAGCCCT CGAGTCAAT  
 10201 ACGTAGTCTG TGCAAGTCCG CACCAGGTAC TGGTATCCCA CCAAAAAGTG CGGCGGCGG  
 10261 TGGCGGTAGA GGGGCCACGG TAGGGTGGCC GGGGTCTCCG GGGCGAGATC TTCCACATA  
 10321 AGGCGATGAT ATCCGTAGAT GTACCTGGAC ATCCAGTGTA TGCCCGCGCG GGTGTGTGAG  
 10381 GCGCGCGGAA AGTCGCGGAC GCGGTTCGAC ATGTTGCGCA CGGCGAAAA GTGCTCCATG  
 10441 GTCGGGACGC TCTGGCGGCT CAGGCGCGCG CAATCGTTGA CGCTCTAGCG TGCAAAAAGA  
 10501 GAGCTGTAA GCGGGCACTC TTCCGTGGTC TGGTGGATAA ATTCCGAAGG GTATCATGCG  
 10561 GGAAGAGCGG GGTTCGAGCC CCGTATCCCG CGCTCGCGCG TGATCCATG GTGTACGCGC  
 10621 CCGGTGTGCA ACCCAGGTGT GCGACGTGAC ACAACGGGG AGTGCTCTCT TTGGCTTCT  
 10681 TCCAGCGCGG CGGCTGCTG CGCTAGCTTT TTGGCCACT GTGCGCGGCG AGGTAAGCG  
 10741 GTTAGGCTGA AAAGCGAAAG CATTAAAGTG CTGCTCTCT GTAGCGGAGG GGTATTTTTC  
 10801 CAAGGTTGTA GTCCGGGAG CCCCCTGTC AGTCTCGGAC CGGCGCGGCG GTGCGGAAAC  
 10861 GGGGTTGGCC TCCCCGTGAT GCAAGACCCC GCTTGCATAA TCCTCCGGAA ACAGGGACGA  
 10921 GCCCTTTTCT TGCTTTTCTG AGATGTACCT GGTGCTCGGG CCGCTCTGCA CCCCTCTGCA  
 10981 CGAGCGGCAA GAGCAAGAGC AGCGGACGAC ATGCAAGGCA CCTCTCCCTC CTCCTACGCG  
 11041 CTCAGAGGGG GCGACATCCG CGGTTGACGC GGCAGGAGT GGTGATTACG AACCCCCGCG  
 11101 GCGCGGGGCC CGGCACTACC TGGACTTGA GAGGGGCGAG GGCCTTGGCG GCGTAGGAGC  
 11161 GCCCTCTCTT GAGCGGTACC CAAGGGTGCA GCTGAAGCGT GATACGCGTG AGGCGTAGCT  
 11221 GCGCGGCGAG AACCTGTTTC GCGACCGCGA CGGAGAGGAG CCGAGGATG TGCGGGATGC  
 11281 AAAGTTCCAC CGAGGGCGCG AGCTGCGGCA TGGCTGTAAT CGCGAGCGGT TGCTGCGGCA  
 11341 GGAGGACTTT GAGCCCGACG CGCGAACCGG GATTAGTCCC GCGCGCGCGC ACGTGGCGCG  
 11401 CGCCGACCTG GTAACCGCAT ACGAGCAGAC GGTGAACGAG GAGATTAACT TTCAAAAAAG  
 11461 CTTTAAACAAC CACGTGCGTA CGCTTGTGGC GCGCGAGGAG GTGGCTATAG CACTGATGCA  
 11521 TCTGTGGGAC TTGTAAAGCG CGCTGAGGCA AAACCCAAAT AGCAAGCCGC TCATGGCGCA  
 11581 GGTGTTCTCT ATAGTGACGC ACAGCAGGGA CACGAGGCA TTCAAGGATG CGCTGCTAAA  
 11641 CATAGTAGAG CCCAGGGGCC GCTGGCTGCT CGATTGTGATA AACATCTG AGAGCAATAGT  
 11701 GGTGCAAGAG CGACAGTTGA CGCTTGTGTA CAAGGTGGCC GGCATCAACT ATTCAATGCT  
 11761 TAGCTTGGGC AAGTTTATAG CCGCGAAGT ATACCATACT CCTTACGTTT CCATAGACAA  
 11821 GGAGTTAAAG ATCGAGGGGT TCTCATGCG CATGGCGCTG AAGGTGTCTT ACTTGAGGCA  
 11881 CGACTTGGGC GTTTATCGCA ACGAGCGCAT CCACAAGGCC GTGAGCGTGA GCGGCGCGCG  
 11941 CGAGCTCAGC GAGCCGAGC TGATGACACG CTGCAAGG CCGCTGCGCTG GCGCGGCGAG  
 12001 CGCGATAGA GAGCGCGAGT CTTACTTTGA CGCGGCGCTG GACCTGCGCT GGGCCCCAG  
 12061 CGAGCGCCCG CTGAGGCGAG CTGGGCGCGG ACCTGGGCTG GCGGTGGCAC CCGCGCGCGC  
 12121 TGGCAACGTC GGGCGGCTGG AGGAATATGA CGAGGACGAT AGTACGAGC CAGAGAGCGG  
 12181 CGAGTACTAA CGGTGATGT TTCTGATCAT ATGATGCAAG ACGCAAGCGA CCGGCGGTG  
 12241 CGGGCGGCGG TCGAGAGCCA CGCGTCCGCG CTTAACTCCA CGAGCAGCTG GCGCGAGGTC  
 12301 ATGGACCGCA TCAATGTGCT GACTGCGCGC AATCTGACCG CGTTCCGCGA GAGCGCGGAG  
 12361 GCCAACCGGC TCTCGCAAT TCTGGAAGCG TGTGTTCCGG CGGCGCGCAA CCGACGAC  
 12421 GAGAGGTTGC TGGCGATGCT AAACCGCGTG CCGGAAAAA GGGCCATCCG GCGCGACGAG  
 12481 GCGCGCTGCG TCTACGAGCG CTGCTTTCAG CGGTGCTGCT GTTAACAAC GCGCAACGTC  
 12541 CAGACCAACC TGGACCGGCT GGTGGGGGAT GTGCGCGAGG CCGTGGGCGA CGGTGAGCGC  
 12601 CGGACGACAG AGGGCAACCT GGGCTCCATG GTTGCACTAA ACGCCCTTCT GATGACAGC  
 12661 CCGGCCAACG TGGCGCGGGG ACAGGAGGAC TACACCAACT TTGTGAGCGC ACTGCGGCTA

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12721 ATGGTGACTG AGACACCGCA AAGTGAGGTG TACCAGTCTG GCCCAGACTA TTTTTCGAG  
 12781 ACCAGTAGAC AAGGCTGCA GACCGTAAAC CTGAGCCAGG CTTTCAAAAA CTTCGAGGGG  
 12841 CTGTGGAGCG TCGGCGCTCC CACAGGCGAC CGCGCGACCG TGTCGTACTT GCTGACGCCC  
 12901 AACTCGGCGC TGTTGCTGCT GCTAATAGCG CCCTTCAAGG ACAATGTCAG CGTGTCCCGG  
 12961 GACACATACC TAGGTCACTT GCTGACACTG CCAATAGTGA CCAATAGTGA GCGCATGTG  
 13021 GACGAGGATA CTTTCCAGGA GATTACAAGT GTACGCGCGC CGCTGGGGCA GAGGACACAG  
 13081 GGCAGCTGAG AGGCAACCTT AAATCACTGT CTGACCAACC GCGCGCAGAA GATCCCTCTG  
 13141 TTGACAGTGT TAAACAGCGA GGAGGAGCGC ATTTTGGCTG ACCTGACAGA GAGCGTGAGC  
 13201 CTTAACTCTG TCGCGGACGG GGTAAACGCG AGCGTGGCGT TGGACATGAC CGCGCGCAAC  
 13261 ATGGAACCGG GCATGTATGC CTCAAACCGG CCGTATTATCA ACCGCTTAAAT GGAATCTCTG  
 13321 CATCGCGCGG CGCGCGTGAA CCGCGAGTAT TTCACCAATG CATCTCTGAA CCGCGACTGG  
 13381 CTACCGCCCC CTGGTTTCTA CACCGGGGGA TTGAGGTTGC CCGAGGGTAA CGATGGATTCT  
 13441 CTCTGGGAGC ACATAGACGA CAGCGTGTCT TCCCGCGAAC CGCAGACCCCT GCTAGAGTTG  
 13501 CAACAGCGCG AGCAGGCGAGA GCGCGCGCTG CGAAAGGAAA GCTTCCGACG CCGAAGCAGC  
 13561 TTGTCCGATC TAGGCGCTGC GCGCGCGCGG TCAGATGTCTA GTAGCCCATT TCCAAGTTTG  
 13621 ATAGGGTCTC TTACAGCAC TCGCACCAAC CGCCCGCGCC TGCTGGGCGA GGAGGAGTAC  
 13681 CTAACAACTC CGCTGCTGCA GCGCGAGCGC GAAAAAAACG TGCTCCGCGC ATTTCCCAAC  
 13741 AACCGGATAG AGAGCTCTAGT GGACAAAGTA AGTATGTGGA AGACCTTGAAG CGAGGAGCAC  
 13801 AGGAGCGTGC CAGGCGCGCG CCGCGCCACC CGTGTCTCAA GGCAAGACGC TCAGCGGGGT  
 13861 CTGGTGTGGG AGGACGATGA CTCGCGCAGC GACAGCAGCG TCTGGTATTG GGGAGGGAGT  
 13921 GCGCAACCCG TTGCGCACTT TCGCCCCAGG CTGGGGAGAA TGTTTAAAAA AAAAAAAGC  
 13981 ATGATGCAAA ATAAAAAACT CACCAAGCGC ATGGCACCGA GCGTTGGTTT TCTTGTATTCT  
 14041 CCGTTAGTAT GCGGCGCGCG GCGATGTATG AGGAAGGTCC TCTCTCCCTCG TACGAGAGTG  
 14101 TGTGTAGCGC GCGCGCAGTG GCGCGCGCGC TGCGTTCTCC CTTCGATGCT CCGCTGGAGC  
 14161 GCGCGTTTGT GCGTCCGCGG TACCTCGCGC CTACCGGGGG GAGAAACAGC ATCCGTACTT  
 14221 CTGAGTTGGC ACCCTATTTC GACACCAACC GTGTGTACTT GTTGGTCAAGT AAGTCAACGG  
 14281 ATGTGGCATC CCTGAATAC CAGAACGACC ACAGCAACTT TCTGACCAAG GTCAATCAA  
 14341 ACAATGACTA CAGCGCGGGG GAGGCAAGCA CACAGACCAT CAATCTTGAC AAGCGGTGCG  
 14401 ACTGGGCGCG CGACCTGAAG ACCATCTTGC ATACCAACAT GCCAAATGAG AACGAGTTCA  
 14461 TGTTTACCAA TAAGTTTAAAG GCGCGGGTGA TGCTGTGCGC CTTGCTTACT AAGGACAATC  
 14521 AGGTGGAGCT GAAATACGAG TGGTGGAGT TCACGCTGCG CGAGGGCAAC TACTCCGAGA  
 14581 CCATGACCAT AGACCTTATG AACACCGCGA TCGTGGAGCA CTACTTGAAA GTGGCGAGAC  
 14641 AGAACGCGGG TCTGGAAAGC GACATCGGGG TAAAGTTTGA CACCGCGAAC TCTCAGATGG  
 14701 GGTTTGACCC CGTCACTGGT CTGTCTATGC CTGGGGTATA TACAAAGCAA GCCTTCCATC  
 14761 CAGACATCAT TTTGCTGCCA GGATGCGGGG TGGAATTCAC CCAACCGCCG CTGAGCAATC  
 14821 TGTTGGGCAT CCGCAAGCGG CAACCCCTCC AGGAGGGCTT TAGGATCAAC TACGATGATC  
 14881 TGGAGGGTGG TAACATTCCC GCACTGTTGG ATGTGGAGCG CTACAGGGCG AGCTTGAAG  
 14941 ATGACACCGA ACAGGGCGGG GGTGGCGCAG CGCGCAGCAA CAGCAGTGGC AGCGCGCGCG  
 15001 AAGAGAACTC CAACGCGGCA GCGCGGCGAA TGCAAGCATG GAGGAGCATG AAGCATCATG  
 15061 CCATTGCGGG CGACACCTTT GCCACACGGG CTGAGGAGAA GCGCGCTGAG CCGGAAGCGG  
 15121 CGCGCGAAGC TGCCCGCCCC GCTGCGCAAC CCGAGGTGAG GAAGACTCAG AAGAAACAGC  
 15181 TGATCAAAAC CCTGACAGAG GACAGCAAGA AACCGAGTTA CAACCTTAAT AGCAATGACA  
 15241 GCACCTTCAC CCAATACCGC AGCTGTGTACC TTGCATACAA CTACGCGCAG CCGTACAGCG  
 15301 GAACTCGCTC ATGGACCTTG CTTTGCACCT CTGACGTAACT CCGCGCGCTG GAGCAGGTCT  
 15361 ACTGTTGCTT GCGACAGATG ATGCAAGACC CGCTGACCTT CCGCTCCAGC CGCGAGATCA  
 15421 GCACTTTCCC GGTGTGTGGC GCGGAGCTGT TGCCCGTGCA CTCCAAGAGC TTCTACAAGC  
 15481 ACCAGGCGGT CTACTCCCAA CTCATCCGCG AGTTTACTCT TCTGACCCAC GTGTCTCAATC  
 15541 GCTTTCCCGA GAACAGAGT TTGGCGCGCC CCGAGCGCCC CACCATGACC CCGTCAAGTG  
 15601 AAAACGTTCC TGCTCTCACA GATCACGGGA CGCTACCGCT CGGCAACAGT ATCGGAGAGG  
 15661 TCCAGCGAGT GACCATTAAT GACGCGCAGC GCGCGACCTG CCGCTACGTT TACAGAGGCC  
 15721 TGGGCAATGT CTGCGCGCGC GTCTTATGGA GCGCGACTTT TTGAGCAAGC ATGTCCATCC  
 15781 TTATATCGCC CAGCAATAAC ACAGGCTGGG CTCTGCGCTT CCGAAGCAAG ATGTTTGGCG  
 15841 GGGCCAAAGAA GCGCTCCGAC CAACACCCAG TCGCGCGTGC CGGCGACTAC CCGCGCGCCT  
 15901 GGGCGCGCGA CAAACGCGCG CGCACTGGGG GCACCAACGCT CGATAGCGCC ATGACGCGCG  
 15961 TGGTGGAGGA GCGCGCAAC TACAAGCCCA CGCGCGCACG AGTGTCCACA GTGGACGCGG  
 16021 CCATTCAAGC GGTGTTGCGC GGAGCCCGCG CCTATGCTAA AATGAAGAGA CCGGCGAGGC  
 16081 GCGTAGACAG TCGCGACCGC GCGCGACCGC GCACTGCGCG CCAACGCGCG CCGCGCGCGC

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FIGURE 22  
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16141 TGCTTAAACCG CGCACGTCGC ACCGGCCGAC GGGCGGCCAT GCGGGCCGCT CGAAGGCTGG  
 16201 CCGCGGGTAT TGTCACGTGT CCCCACAGGT CCAGGCGACG AGCGGCGCGC GCAGCAGCCG  
 16261 CGGCGATTAG TGCTATGACT CAGGGTCGCA GGGGCAACGT GTATTGGGGT CGCGACTCGG  
 16321 TTAGCGGCGCT CGCGCTGCCG GTGCGCACCC GCCCCCCGCG CAACTAGATT GCAGAAGAAA  
 16381 ACTACTTAGA ATCTGACTGT TGTATGTATC CAGCGCGCAAC GCGCGCGCAAC GAAGCTATGT  
 16441 CCAAGCGCAA AATCAAGAA GAGATGCTCC AGGTTCATCG CCGCGAGATCT TATGGCCCCC  
 16501 CGAAGAAGGA AGAGCAGGAT TACAAGCCCC GAAAGCTAAA GCGGGTCAA AGAAGAAAAGA  
 16561 AAGATGATGA TGATGAACCT GACGACGAGG TGGAGCTGCT GCACGCTACC GCGCCGAGCG  
 16621 GACGGGTACA GTGGAAAGGT CGACGCGTAA AACGTGTTTT GCGACCGCGC ACCACCGGTG  
 16681 TCTTTACGCC CGGTGAGCGC TCCACCOCGA CCTACAAGCG CGTGTATGAT GAGGTGTACG  
 16741 GCGACGAGGA CTTGCTTGA GCGGCCAACG AGCCCTCGGG GGAGTTTGGC TACGGAAGCG  
 16801 GGCATAAGGA CATGCTGGCG TTGCGCGCTG ACAGGGGCAA CCGAACACCT AGCTTAAAGC  
 16861 CGGTAAACAT GCAGCAGGTG CTGCGCGCGC TTGACCCGTC GGTACCCAA GCGCGCGCTAA  
 16921 AGCGGAGTCT TGGTGACTTG GCACCCACCG TCAGCTGAT GGTACCCAA GCGCGCGCTAA  
 16981 TGGAAAGATG CTTGGAAGAA ATGACCGTGG AACTCGGCT GGTACCCAA GCGCGCGCTAA  
 17041 GCGCAATCAA GCAGGTGGCG CCGGACTGCG GCGTGACAGC CGTGGAGCTT CAGATACCCA  
 17101 CTACGAGTAG CACCAAGTAT GCCACCGCCA CAGAGGGCAT GGAGACACAA ACTGACCCCG  
 17161 TTGGCTCAGC GGTGGCGGAT GCGCGGTGCG AGCGGTGCGC TGCGGCGCGC TCCAGAGCTT  
 17221 CTACGAGAGT GCACAAAGGAC CCGTGGATGT TCGCGTTTTT AGCCCCCGCG CGCCCGCGCG  
 17281 GTTCGAGGAA GTACGCGGCC GCCAGCGCGC TACTGCGCGA ATATGCTTCA CACTCTTCCA  
 17341 TTGCGCTTAC CCCCAGCTAT CGTGGCTACA CCTACGCGCC CAGAAGACGA GCACTACCC  
 17401 GACGCGGAAC CACCACTGGA ACCCGCGCGC GCGCTGCGCG TCGCGAGCGA GCTGTGCGCC  
 17461 CGATTTCCTG CGCGCAGGTT GCTCGCGAAG GAGGACGAGC CCTGGTCTG CCAACGAGCG  
 17521 GCTACCAACC CAGCATCGTT TAAAGCCGCG TCTTTGTGGT TCTTGACAGT AGAGCCCTCA  
 17581 CTTGCGCGCT CGGTTCCCG GTGCGGGGAT TCCGAGGAAG AATCGACCGT AGGAGGGGGA  
 17641 TGGCGCGCCA CGGCTGACG GCGCGCATCG GTCTGCGCG CACCGCGCGC CGCGCGCGCT  
 17701 CGCACCGGTG CATGCGCGCG GGTATCTGCG CCCTCTTAT TCCACTGATC GCGCGCGCGA  
 17761 TTGCGCGCTG CCGCGGAATT GCATCGGTGG CCTTGACGCG GCAGAGAGCA TGATTAAAAA  
 17821 CAAGTTGCGT GTGGAAGAAAT CAAATAAAAA AGTCTGGAAT CTACGCTCG CTGTGCTCTG  
 17881 TAACATTTTT GTAGAATGGA AGACATCAAC TTTGCGTCTC TGCGCCCGCG ACACGCGCTG  
 17941 CGCCCGTTCA TGGGAAACTG GCAAGATATC GGCACGACA ATATGAGCGG TGCGCGCTTC  
 18001 AGCTGGGGCT CGCTGTGGAG CGGCATTAAA AATTTGCGTT CCACCGTTAA GAACATATGGC  
 18061 AGCAAGCGCT GGAACAGCAG CACAGGCGAG ATGCTGAGGG ATAAGTTGAA AGAGCAAAAT  
 18121 TTCCAACAAA AGGTGGTATA TGGCGTGGCC TCTGGCATTA GCGGGGTGCT GGAACCTGGCC  
 18181 AACCAGGCG TGCAAAATAA GATTAAACAGT AAGCTTGATC CGCGCGTTCG CTAGAGGAG  
 18241 CCTCCACCGG CGGTGGAGAC AGTGTCTCCA GAGGGGCGTG GCGAAAAGCG TCGCGCGCCC  
 18301 GACAGGGGAG AAACCTGTGT GACGCAATA GACGAGCTC CCTGTACGA GAGGACGACT  
 18361 AAGCAAGGCC TGCCCAACAC CCGTCCCATC GCGCCCATGG CTACCGAGAT GCTGGGCGAG  
 18421 CACACACCGG TAACGCTGGA CCTGCTCCC CCGCGGACA CCGAGCAAG ACTGTGCTG  
 18481 CCAGGCGCGA CGCGCGTTGT TGTAAACCGT CCTAGCGCG CGTCCCTGG CCGCGCGCGC  
 18541 AGCGGTCCCG GATCGTTGCG GCCCGTAGCC AGTGGCACT GCGCAAGCAC GAGTGAAGAG  
 18601 ATCTGGGGTG TGGGGGTGCA ATCCCTGAAG CGCGCAGCAT GCTTCTGAAT AGCTAAGCTG  
 18661 TGTATGTGT GTCATGTATG GTCCATGTC GCGCCAGAG GAGCTGTGAG ACTGACGAGC  
 18721 GCGCGCTTTC CAAGATGGCT ACCCTTCGA GTATGCCGA GTGGTCTTAC ATGCAATCT  
 18781 CCGGCGAGGA CGCTCGAGT TACCTGAGCG CCGGCTGTGT CGAGTTTGGC CGCGCACGCG  
 18841 AGACGTACTT CAGCTGGAAT AACAGTTTAA GAAACCCAC GGTGGCGCTT ACACGACGAG  
 18901 TGACCAACGA CCGGTCCAGC CGTTTGACCG TACGCTGGGG TGATAACCGT GTGCTGAGCA  
 18961 CTGCTACTCT GTACAAGGCG CGGTTACCCC TAGCTGTGGG TGATAACCGT TTTAGGCCCT  
 19021 TGGCTTCCAC GTACTTTGAC ATCCGCGGCG TGCTGTGACG GGGCCCTACT TTTAGGCCCT  
 19081 ACTCTGGGAC TGCTTACAC CCGCTGGCTC CCAAGGGTGC CCAAGCTTCT TGCGAATGGG  
 19141 ATGAAGCTCG TACTGCTCTT GAAATAAAC TAGAAGAAGA GGACGATGAC AACGAGAGAG  
 19201 AAGTAGACGA GCAAGCTGAG CAGCAAAAAA CTACGATATT CCAAGCGAGC CTCTATTCTG  
 19261 GTATAAATAT TACAAGGAGG GGTATTCAAA TAGGTGTGCA AGGTCAACAG CCTAATATAT  
 19321 CGGATAAATC ATTTCAACCT GAACCTCAAA TAGGAGAATC TAGGTGTGAC GAACCTGTAA  
 19381 TTAATCATGC AGCTGGGAGA GTCTTTAAAA AGACTACCCC AATGAACACA TTTTACGTTT  
 19441 CATATGCCAA ACCCAAAAT GAAATGAGG GCAAGGCAAT TCTTGTAAAG CACAAAAATG  
 19501 GAAAGCTAGA AAGTCAAGTG GAAATGCAAT TTTTCTCAAC TACTGAGGCG ACCGACGCGA

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19561 ATGGTGATAA CTGACTCCT AAAGTGGTAT TGTACAGTGA AGATGTAGAT ATAGAAACCC
19621 CAGACACTCA TATTTCTTAC ATGCCCACTA TTAAGGAAGG TAACTCACGA GAACTAATGG
19681 GCCAACAACT TATGCCCAAC AGGCCATAAT ACATTGCTTT TAGGGACAA TTTATTGGTC
19741 TAATGTATTA CAACAGCACG GGTAAATATG GTGTCTTGCG GGGCCAAGCA TCGCAGTTGA
19801 ATGCTGTTGT AGATTTGCAA GACAGAAACA CAGAGCTTTT ATACCAGCTT TGTCTTGATT
19861 CCAATGGTGA TAGAACCAGG TACTTTTCTA TGTGGAATCA GCGTGTGAC AGCTATGATC
19921 CAGATGTTAG AATTATTGAA AATCATGGAA CTGAAGATGA ACTTCCAAAT TACTGCTTTT
19981 CACTGGGAGG TGTGATTAA TACAGAGACT TTACCAAGGT AAAACCTAAA ACAGGTCAGG
20041 AAAATGGATG GGAAGAAAGT GCTACAGAAT TTTACAGATA AAATGAAATA AGATTGTGAA
20101 ATAAATTTGC CATGGAATC AATCTAAATG CCAACCTGTC GAGAAATTTT CTGTACTCCA
20161 ACATAGCGCT GTATTTGCCG GACAAGCTAA AGTACAGTCC TTCCAACTGT AAAATTTCTG
20221 ATAAACCCAA CACCTACGAC TACATGAACA AGGAGTGGT GGCCTCCGGG TTAGTGGACT
20281 GCTACATTAA CCTTGAGCA CGCTGGTCCC TTGACTATAT GGACAACTGT AACCCATTTA
20341 ACCACCCAGC CAATGCTGGC CTGCGCTACC GCTCAATGTT GCTGGGCAAT GGTGCTATG
20401 TGCCCTTCCA CATCCAGGTG CCTCAGAAGT TCTTTGCCAT TAAAAACCTC TTCTCCTGTC
20461 CGGCTCTATA CACCTACGAG TGAACCTTCA GGAAGGATGT TAACATGGTT CTGACAGACT
20521 CCCTAGGAAA TGACCTAAGG GTTGACGGAG CCAGCATTA GITTGTATAG ATTTGCTTTT
20581 ACGCCACCTT CTTCGCCATG GCCCAACACA CGGCTCCAC GCTTGAAGCC ATGCTTAGAA
20641 AGACACACCA CGACCAGTCC TTAAACGACT ATCTCTCCGC CGCCAACTGT CTCTACCTTA
20701 TACCOCGCCA CGCTACCAAC GTGCCCATAT CCATCCOCTC CCGCACTGG GCGGCTTTCC
20761 GCGGCTGGGC CTTCAGCGCG CTTAAGACTA AGGAAACCCG ATCACTGGG TGGGCTACGG
20821 ACCCTTATTA CACTACTCT GGTCTATATC CTTACTAGA TGAACCTTTT TACTCAACC
20881 ACACCTTTAA GAAGGTGGCC ATTACCTTTG ACTCTTTCTG CAGCTGGCTT GGCATGACC
20941 GCTGCTTCAA CCCCACGAG TTGAAATTA AGCGCTCAGT TGACGGCAAG GTTACAAAG
21001 TTGCCAAGTG TAACATGACC AAAGACTGGT TCCTGGTACA AATGCTAGCT AACTACACAA
21061 TTGGCTACCA GGGCTTCTAT ATCCACAGA CGCTACAAGA CCGCATGTAC TCCTTTCTTA
21121 GAAACTTCCA GCCCATGAGC CGTCAGTGG TGGATGATAC TAAATACAG GACTACCAAC
21181 AGGTGGGCAT CCTACACCAA CACAACAAC TCTGGATTGT TGGCTAGTAC CCCCCACCA
21241 TGCGCGAGG ACAGGCGTAC CTGCTAATC TCOCCTATCC GCTTATAGGC AAGACCGCAG
21301 TTGACAGCAT TACCAGAAA AAGTTTCTTT GCGATCGCAC CTTTGGGCG ATCCCATCTT
21361 CCACTAATCT TATGTCATG GCGCATCA CAGACTGGG CCAAAACCTT CTCTACGCCA
21421 ACTCGCCCCA CGCGCTAGAC ATGACTTTTG AGGTGGATCC CATGGACGAG CCCACCTTTC
21481 TTTATGTTGT GTTTGAAGTC TTGACGTGG TCGGTGTGCA CCGCGCGCAC CCGGCGTCA
21541 TCGAAACCGT GTACCTGCGC AGCGCTTCT CCGCGCGCAA CGCCACAA CA TAAAGAACCA
21601 AGCAACATCA CAACAGCTG CGGCCATGGG CTCAGTAG CAGGAACCTA AAGCATGTT
21661 AACAGACTCT GGTGTGGGC CATATTTTGT GGCACCTAT GACAAGCGCT TTCCAGCTTT
21721 TGTTCCTCCA CACAAGCTCG CCTGCGCAT AGTCAATAG CCGGTGCGG AGACTGGGG
21781 CGTACACTGG ATGCGCTTTG CCTGGAACCC GCATCTCAA ACATGCTACC TCTTTAGCC
21841 TTTGGCTTT TCTGACCAG GACTCAAGCA GGTTTACCAA GTTGAATGAC AGTCACTCTT
21901 GCGCGTAGC GCAATTGCTT CTTCGCCGA CGGTGTATA ACGTGGAAA AGTCCACCA
21961 AAGCGTACAG GGGCCCACT CCGCGCGCTT TGCTGACTTT TGCTGACTGT TCTTCAAGC
22021 CTTTGGCCAC TGGCCCCAAA CTCCTAGGA TCACAAACCC ACCATGACC TTATTACGG
22081 GGTACCCAC TCCATGCTCA ACAGTCCCCA GGTACAGCG CCGCTGCTC GTACACAGA
22141 ACAGCTCTAC AGCTTCTGG AGCGCACT GCCTTACTT CGCAGCCACA GTGCGCAGAT
22201 TAGAGAGCGC ACTTCTTTT GTCACTTGAA AAATATGTA AAATAATGTA CTGAGACAC
22261 TTCAATATA GGCAAATGCT TTTATTTGTA CACTCTCGG TGATTTATTA CCCCCACCT
22321 TGCCGCTGCG GCGGTTTAAA AATCAAAGG GTTCTGCGC GCATGCTAT GCGCCACTG
22381 CAGGACACG TTGCGATCT GGTGTTTAGT GCTCCACTTA AACTCAGGCA CAACCATCG
22441 CGCGAGCTCG GTGAAGTTT CACTCCACG CGTCCGACG GTTCCACAG CGTTTAGAC
22501 GTGCGGCGCC GATATCTGA AGTGCAGTT GGGGCTCCG CCGTGGCGC GCGAGTTGG
22561 ATACACAAGG TTGACGACT GGAACACTAT CAGCGCGAG TGGTGCAGC TGCCAGCAC
22621 GCTCTTGTCG GAGATCAGAT CCGGCTCCG GTCTCTCGC TTGCTCAGG CGAAGGAGT
22681 CACTCTTGTG AGCTGCCTTC CCAAAAAGG CGGTGCCCC GCTTCTAGG TGACTCGCA
22741 CCGTAGTGGC ATCAAAGGT GACCGTCCC GGTCTGGCG TTAGATACA GCGCTGATC
22801 AAAAGCCTTG ATCTGCTTAA AAGCCACTG AGCCTTTGCG CCTCAAGA AGACATGCG
22861 GCAAGACTGG CCGGAAACT GATTGCGCG ACAGGCCCG TCGTGCAGC AGCACCTTG
22921 GTGCGGTG TGAGATCTGA CCACAATTG GCCCACCAG TTCTCACGA TCTTGGCCTT

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FIGURE 22  
(SHEET 7)

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22981 GCTAGACTGC TCCTTCAGCG CGCGCTGCCG GTTTTCGCTC GTCACATCCA TTTCAATCAC
23041 GTGCTCCTTA TTTATCATAA TGCTTCGCTG TAGACACTTA AGCTCGCCTT CGATCTCAGC
23101 GCAGCGGTGC AGCCACAACG CGCAGCCCGT GGGCTCGTGA TGCTTTGAGG TCACCTCTGC
23161 AAACGACTCG AGGTACGCCT GCAGGAATCG CCCCATCATC GTCAACAAAGG TCTTGTGTCT
23221 GTGTGAAGTC AGCTCGAACC CGCGGTGCTC CTGCTTCAGC CAGGCTTTGC ATACGCCCGC
23281 CAGAGCTTCC ACTTGGTCAG GCAGTAGTTT GAAGTTGCGC TTTAGATCGT TATCAGCGTG
23341 GTACTTTGTC ATCAGCGCGC GCGCAGCCTC CATGCCCTTC TCACAACGAG ACAGACTTCG
23401 CACACTCAGC GGGTTCATCA CCGTAATTTC ACTTTCGCTT TCCTCGGGCT CTTCCTCTTC
23461 CTCTTGGCTG CGCATACCAC GCGCCACTGG GTCGTCTTCA TTCAGCCGCC GCACGTGTGC
23521 CTTACTCTCT TTGCCATGCT TGATTAGCAC CGGTGGGTGT CTGAACCCCA CCAATTGTAG
23581 CGCCACATCT TCTCTTTCTT CCTCGCTGTC CACGATTACC TCTGTGTATG CGGGCGGCTC
23641 GGGCTTGGGA GAAGGGGCGT TCTTTTCTT CTGTGGCGCA ATGGCCAAAT CGCCGCGCGA
23701 GTCTCATGGC CGCGGGCTGG GTGTGCGCGG CACCAGCGCG TCTTGTGTATG AGTCTTCTCT
23761 GTCTTCGGAC TCGATACGCC GCCTCATCCG CTTTTTTGGG GCGCGCCGGG GAGGCGCGCG
23821 CGACGGGGAG GGGGACGACA CGTCTCCATC GTTGTGGGGA CGTCCGCGCG CACCCGCTTC
23881 GCGCTCGGGG GTGGTTTGGC GCTGCTCTCT TTTCCGACTG GCCATTTCTT TCTCTATAG
23941 GCAGAAAAG ATCATGGAGT CAGTCGAGAA GAAGGACAGC CTAACCGCCC CCTCTAGATT
24001 CGCCACCACC GCCTCAACCG ATGCCGCCAA CGCGCTACCC ACCTTCTCCG TCGAGGACCC
24061 CCGCTCTGAG GAGGAGGAAG TGATTATCGA GCAGGACCCA GGTTTTGTAA CGGAGACAGA
24121 CGAGAGCCGC TCAGTACCAA CAGAGGATAA CAGGACAAAG CAGAGGCCAA GAGAGGCCAA
24181 CGAGGAACAA CTGGGGCGGG GGGACGGAAG GCGATGGGAC TACCTAGATG TGGGAGACGA
24241 CGTCTGTTG AAGCATCTGC AGCGCCAGTG CCCTATTATC TGCGACCGGT TGCAGAGAGC
24301 CAGCGATGTG CCCCTCGCCA TAGCGGATGT CAGCTTTGCC TAGAACCGCC ACCTATTCTC
24361 ACCTCGGTGA CCCCCCAAA GCGCAAGAAA CGGCACATGC GAGCCCAAGC AGCGCTCTAA
24421 CTCTACGCC GTATTGTCGG TGCCAGAGGT GCTTGCACCC TATCACATCT TTTTCCAAAA
24481 CTGCAAGATA CCCCTATCTT CGCGTGCCAA CGCGCAGCCG CACTGCGGCT AGCTGGGCTT
24541 GCGGCAGGGC GCTGTCTATC CTGATATCGC CTGCTCAAC GAAGTGCCAA AAATCTTTGA
24601 GGGCTTTGGA CGCGACGAGA AGCGCGCGCG AAACGCTCTG CAACAGGAAA ACAGCGAAAA
24661 TGAAAGTCAC TCTGAGATGT TGTGTGAAGT CGAGGGTGAC AACGCGCGCC TCCCGGATG
24721 AAACGCGAGC ATCGAGGTCA CCCACTTTGC CTACCCGGCA CTTAACTCAC CCCCCAAGT
24781 CATGAGCACA GTCATGAGTG AGCTGATCGT GCGCCGTGCG CAGCCCTCTG AGAGGATGC
24841 AAATTTGCAA GAACAAACAG AGGAGGCGCT ACCCGCATTT GCGCATGCGG AGCTAGCGCG
24901 CTGGCTTCAA ACGCGCAGGC CTGCCGACTT GGAGGAGCGA CGCAAACTAA TGATGGCCGC
24961 AGTGTCTGTT ACCGTGGAGC TTGAGTGATC GCAGCGGTTT TTTGCTGACC CGGAGATGCA
25021 CGGCAAGCTA GAGGAAACAT TGCACTACAC CTTTCGACAG GGTACGTATC GTCAGGCTGT
25081 CAAGATCTCC AACGTGGAGC TCTGCAACCT GGTCTCTTAC CTTGGAATTT TGCAAGAAAA
25141 CCGCCTTGGC CAAAACGTGC TTCAITCCAC GCTCAAGGGC GAGGCGCGCC CAGAGTACGT
25201 CGCGCACTGC GTTTACTTAT TTCTATGCTA CACCTGGCAG ACGGCCATG GCGTTTGCGA
25261 CGAGTGCTTG GAGGAGTGCA ACCTCAAGGA GCTCGAGAAA CTTGCTAAGC AAAAATCTGA
25321 GGACTATGAG ACGGCCCTTCA ACGAGCGCTC CGTGGCGCGG CACCTGGGCG ACATCATTTT
25381 CCCCCAAGCG CTGCTTAAAA CCGTGCAACA GGGTCTGCCA GACTTCAACA GTCAAGCAT
25441 GTTGCAAGAC TTTAGGAAGT TTATCTTAGA CGGCTCAGGA ATCTTGCCCG CCACCTGTCT
25501 TGCACTTCTT AGGCACTTTG GTGCCATTAA GTACCGGAA TGCCTCTCCG CGCTTTGGGG
25561 CCACTGTCTC CTCTGCGAGC TAGCCAAGTA CCTTGCTTAC CACTCTGACA TAATGGAAGA
25621 CTTGAGCGGT GAGCGTCTAC GTGAGTGTCA CTTGCTCTGC AACCTATGCA CCCCAGACCG
25681 CTCCCTGGTT TGCAATTTCG AGCTGTCTAA CGAAAGTCAA ATTTACGCTA CTTTGGAGCT
25741 CCAAGGTCCT TCGCTTGAGC AAAAGTCCGC GGCTCGGGGG TGGAACTCTA CTTCCGGGCT
25801 GTGGACGTG GCTTACCTTC CCAAATTTGT ACCTGAGGAC TACCACGCCG ACGAGATTAG
25861 GTTCTACGAA GACCAATCCC GCGCGCCAAA TGCGGAGCTT ACGCTCTGCG TCATTACCCA
25921 GGGGCCACAT CTTGGCCAAAT TGCAAGCCAT CAACAAAGCC CGCCAGAGT TTCTGCTACG
25981 AAAGGGACGG GGGGTTTACT TGACCCCCCA GTCCGGCGAG GAGCTCAAGC CAATCCCGCC
26041 CGCGCGCAG CCGTATCAGC AGCAGCGCGG GCGCTTGTGT TCCGAGATG CACCCAAAAA
26101 AGAAGCTGCA GCTGCCCGCG CCACCCACGG ACGAGGAGGA ATACTGGGAG AGTCAGGCGC
26161 AGGAGGTTTT GGACGAGGAG GAGGAGGACA TGATGGAAGA CTGGGADAGC CTAGACGAGG
26221 AAGCTTCCGA GGTGGAAGAG GTGTGAGAGC AAACACCGTC ACCCTCGGCT GCATTCCCCCT
26281 CGCCGGCGCC CAGAAATGCG GCAACCGGTT CACACCTTGG GCTCTCTCAG GGAACCAGGG
26341 CGCCGCGCGC ACTGCCCGTT CGCGACCCA ACGGTAGATG GGACACCACT GGAACCAGGG

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26401 CCGGTAAAGTC CAAGCAGCCG CCGCCGTTAG CCCAAGAGCA ACAACAGCGC CAAGGCTACC  
 26461 GCTCATGGCG CGGGCACAAG AACCGCATAG TTGCTTGCTT GCAAGACTGT GGGGGCAACA  
 26521 TCTCTTTGGC CCGCCGCTTT CTCTCTACCC ATCAGCGCGT GGCCTTCCCC CGTAACATCTC  
 26581 TGCATTACTA CGTCACTCTC TACAGCCCAT ACTGCAACCGG CGCGACGCGC AGCGCGCAGCA  
 26641 ACAGCAGCGG CCACACAGAA GCAAAAGCGA CGGATAGCA AGACTCTGAC AAAGGCCAAG  
 26701 AAATCCACAG CGCGCGGCAG AGCAGGAGGA GGAGCGCTGC GTCTGGCGCC CAACGAACCC  
 26761 GTATCGACCC GCGAGCTTAG AAACAGGATT TTTCCTACTC TGATATCTAT ATTTCAACAG  
 26821 AGCAGGGGCC AAGAACAGA GCTGAAATA AAAACAGAGT CTCTGCGATT CCTCACCOCG  
 26881 AGCTCGCTGT ATCACAAGAG CGAAGATCAG CTTCGGCGCA CGCTGGAAAG CGCGGAGGCT  
 26941 CTCTTCAGTA AATACTGGCG GCTGACTCTT AAGGACTAGT TTGCGGCCCT TTCTCAAAAT  
 27001 TAAGCGCGAA AACTACGTCA TCTCCAGCGG CACACCCCGG CGCCAGTCAAG GTTCGTCAAG  
 27061 GCCATTATGA GCAAGGAAAT TCCACGCCCC TACATGTGGA GTTACACAGC ACAATGGAAG  
 27121 CTTCGGCGTG GAGCTGCCCA AGACTACTCA ACCCGAATAA ACTACATGAG CGCGGGACCC  
 27181 CACATGATAT CCGCGGTCAA CGGAATCCCG GCCCACGGA ACCGAATTTCT CTGGAACAG  
 27241 GTTCAGTCTA CCACACACCC TCGTAATAAC CTTAATCCCC GTAGTTGGCC CGCTGCGCTG  
 27301 GTGTACACAG AAGTCCCGC TCCCAACACT GTGGTACTTC CCAGAGACGC CAGGCGGAA  
 27361 GTTCAGATGA CTAACTCAGG GCGCGAGCTT GCGGGCGGCT TTGCTCAGAC GGTGCGGTG  
 27421 CCGGGGACAG GTATAACTCA CTGACAAATC AGAGGGGCGAG GTATTGAGT CACAGACGAG  
 27481 TCGGTGAGCT CCTCGCTTGG TCTCCGTCCG GACGCGACAT TTCAGATGCG CGCGCGCGCG  
 27541 CGTCTCTCAT TCAAGCCTCG TCAGGCAATC CTAACCTCTG AGACTCTGCT CTCTGAGCCG  
 27601 CGCTCTGAGT GCATTGGAAC TCTGCAATTT ATTGAGAGT TTGTGCCATC GGTCTACTTT  
 27661 AACCCCTTCT CGGAGCTCCC CGGCCACTAT CGGATCAAT TTATTCTCAA CTTTGACCGG  
 27721 GTAAGAGTCT CGGCGAGCGG CTCAGACTGA TAAATTAGTG GAGAGGCGCA GCAACTGCGC  
 27781 CTGAAACACG TGGTCCACTG TCGCCGCCAC AAGTCTTTTG CCGCGGACTC CGGTGAGTTT  
 27841 TGCTACTTTG AATTGCCCGA GGATCATATC GAGGATCTTT GTTGCCATCT CTGTGCTGAG  
 27901 TATAATAAAT ACAGAAATTA AAATATACTG GGGCTCTCAT CGCATTCTCT TTAAGCGCAC  
 27961 CGTCTTCAAC CGCCCAAGCA AACCAGGGCG AACCTTACCT GGTACTTTTA ACATCTTCTC  
 28021 CTCTGTGATT TCAACAGATT TCAACGAGAG CGGAGTGAGT TACTAGGAGA ACCTCTCCGA  
 28081 GCTCAGCTAC TCCATCAGAA AAAACACAC CCTCTTACC TGCCGGGAAC GTACCCCTTA  
 28141 TTAAGAAGTA GGCTTCTTGG ATGTGAGCAT CTGACTTTGG CCAGCACCTG TCCCGCGGAT  
 28201 TTGTTCCAGT CCAACTACAG CGACCCACCC TAACAGAGAT GACCAACACA ACCAAGCGCG  
 28261 CCGCCGCTAC CGGACTTACA TCTACCACAA ATACACCCCA AGTTTCTGCG TTTGTCAATA  
 28321 ACTGGGATAA CTGCGGCATG TGGTGGTTCT CCATAGCGCT TATGTTTGTA TGCCTTATTA  
 28381 TTAATGTGCT CATCTGCTGC CTAAGCGCGA AACGCGCCCG ACCACCCATC TATAGTCCCA  
 28441 TCATTGTGCT ACACCCAAAC AATGATGGAA TCCATAGATT GAGCGGACTG AAACACATGT  
 28501 TCTTTTCTCT TACAGTATGA TTAATGAGA TTAATTAGG AATTCTGCTC GAGTTTATTC  
 28561 AGCAGCACCT CTTTGCCCTC CTCCGAGCTC TGGTATTGCA GCTTCTCTCT CGGCGCAAC  
 28621 TTCTCTCACA ATCTAAATGG AATGTGAGTT TCTCTCTGTT CCTGTCCATC GGCACCCATC  
 28681 ATCTTCATGT TGTTCAGAT GAAAGCGCGA AGACCGCTGT AAGATACCTT CAACCCCGTG  
 28741 TATTCATATG ACACGGAAAC CGGTCTCTCA ACTGTGCTTT TTCTTACTCT TCCCTTTGTA  
 28801 TCCCCCAATG GGTTTCAAGA GAGTCCCCCT GGGGTACTCT CTTTGCGCTC ATCCGAACTC  
 28861 CTAGTTACTT CCAATGGCAT GCTTGGGCTC AAAATGGGCA ACGCCCTCTC TCTGGAAGAG  
 28921 CGCGGCAACC TTACTCTCCA AAATGTAACT ACTGTAGACC CACCTCTCAA AAAACCAAG  
 28981 TCAACATAA ACTCTGGAAT ATCTGCAACC CTCAGATGCT CTTCAAGTGT CCAACTGTG  
 29041 GCTGCGCGCG CACCTCTAAT GGTGCGCGCG AACACACTCA CCAATGCAATC ACAGGCCCGG  
 29101 CTAAACGTCG ACAGATCCAA ACTTAGATT GCCACCCAAG GACCCCTCAC AGTGTACAG  
 29161 GGAAGCTAG CCGTGCACAC ATCAGGCCCT CTCACACCA CCGATAGCAC TACCTTAACT  
 29221 ATCATGCTCG CACCCCTCTT AACTACTGCG ACTGGTAGCT TGCGGATCTA CTGGAAGAG  
 29281 CCAATTTATA CACAAAATGG AAAACTAGGA CTAAAGTAGG GGTCCAGGTT TGACTATTAA  
 29341 GAGCACTTAA ACATCTTGAC CGTAGCACTT TTTGATTGAT AGCTAGACGC TTATACTTGA TGTTAGTTAT  
 29401 TTGCAAACTA AAGTTACTGG AGCCTTGGGT TTTGATTGAT AAGGCAATAT GCTACTTAA  
 29461 GTAGCAGGAG GACTAAGGAT TGATTCTCAA ACACAGACGC TTATACTTGA TGTTAGTTAT  
 29521 CGGTTTATGT CTCAAAACCA ACTAAATCTA AGACTAGACG AGGCGCTCTG TTTTATAAAC  
 29581 TCAGCCACCA ACTTGGATAT TAACTACAA ACAGGCCGTT ACTTGTGTTT AGCTCTAAAC  
 29641 AATTCCAAAA AGCTTGAGGT TAACTTAAGC ACTGCCAAAG GGTGATGTTT TGATCTACA  
 29701 GCCATAGCCA TTAATGCAGG AGATGGGCTT GAATTTGGTT CACCTAATGC ACCAACAACA  
 29761 AATCCCTCTA AAACAAAAT TGGCATGGC CTAGAAATTTG ATTCAACAA CGCTATGGTT

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29821 CCTAAACTAG GAACTGGCCT TAGTTTTGAC AGCACAGGTT CCATTACAGT AGGAAACAAA  
 29881 AATAATGATA AGCTAACTTT GTGGACACCA CCAAGTCCAT CTCCTAACTG TAGACTAAAT  
 29941 GCAGAGAAAG ATGCTAAACT CACTTTGGTC TTAACAAAAT GTGGCAGTCA AATACTTGCT  
 30001 ACAGTTTTCAG TTTTGGCTGT TAAAGGCAGT TTGGCTCCAA TATCTGGAACT AGTTCAAAGT  
 30061 GCTCACTTTA TTATAAGATT TGACGAAAAT GGAGTGTCTT TAAACAATTC TCTTCTGGAC  
 30121 CCAGAATATT GGAACCTTTAG AAATGGAGAT CTTACTGAAG GCACAGCCTA TACAAAACGT  
 30181 GTTGGATTTA TGCCTAACCT ATCAGCTTAT CCAAAATCTC ACGGTAAAGT TGCCAAAAGT  
 30241 AACATTGTCA GTCAAGTTTA CTTAAACGGA GACAAAACCT AACCTGTAACT ACTAACCAAT  
 30301 ACACATAACG GTACACAGGA AACAGGAGAC ACAACTCCAA GTGCATACTC TATGTCAITT  
 30361 TCATGGGACT GGTCTGGCCA CAATACATT AATGAAATAT TTGCCACACT CTCITACACT  
 30421 TTTTCATACA TTGCCCAAGA ATAAGAATG TTTTGTGTTA TGTTTCAACG TGTTTATTIT  
 30481 TCAATTGTCAG AAAATTTCAA GTCAATTTTC ATTCAAGTAG ATAGCCCCAC CACCAATAGT  
 30541 CTTATACAGA TCACCGTACC TTAATCAAAC TCACAGAACC CTAGTATTCA ACCTGCCACC  
 30601 TCCCTCCCAA CACACAGAGT ACACAGTCC TCTCCCCGGT CTGGCCTTAA AAAGCATCAT  
 30661 ATCATGGGTA ACAGACATAT TCTTAGGTGT TATATTCCAC ACGTCTTCTC GTGAGCCCAA  
 30721 CAGCTCATCA GTGATATTAA TAAACTCCCC GGGCAGCTCA CTTAAGTTCA TGTGCTGTCT  
 30781 CAGCTGCTGA GCCACAGGCT GCTGTCTCAAC TTGCGTGTGC TTAACGGGGC GCGAAGGAGA  
 30841 AGTCCACGGC TACATGGGGG TAGAGTCAAT ATCGTGCACT AGGATAGGGC GGTGTGTGCT  
 30901 CAGCAGGGCG CGAATAAACT GCTGCCGGCG CCGCTCCGCT CTGCAGGAAT TCAACATGGC  
 30961 AGTGCTCTCC TCAGCGATGA TTGCGACCGC CCGCAGCTATA AGGCGCTTGT ACCTCCGGGC  
 31021 ACACAGCGCG ACCCTGATCT CACTTAAATC AGCAGCATAA CTGCAGCACA GCACACAAAT  
 31081 ATTTTTCAAA ATCCCAAGT GCAAGGGCGT GTATCCAAAG CTGATGGCGC GACCCACAGA  
 31141 ACCCAGCGTG CCATCATACC ACAAGCGCAG GTAGATTAAAG TGCGACCCCT CATTAACACT  
 31201 CTGCGACATA AACATTACCT CTTTGGCAT GTTGTAACTT ACCACTCCCC GTCACCATAT  
 31261 AAACCTCTGA TTAACATAGG CGCCATCCAC CACCATCTCA AACCACTGGG CCAAAACCTG  
 31321 CCGCGCGGCT ATACACTGCA GGGAAACGGG ACTGGAAACA TGACACCTGA GACCCAGGTA  
 31381 CTGTAACACA TGGATCATCA TGCTCGTCAT GATATCAATG TTGGCACAAC ACAGGACACA  
 31441 GTGCATACAC TTCTTCAGGA TTACAAGCTC CTCCCGGTTT AGAACCTGTA CCGAGGAAC  
 31501 AACCATTCCT TGAATCAGCG TAAATCCCACT ACTGCAGGGA AGACCTCCGA CTGAATCAC  
 31561 GTTGTGCATT GTCAAAGTGT TACATTGGG CAGCAGCGGA TGATCTCCGA GTATGGTAGC  
 31621 CGGAGTTTCT GTCTCAAAG GAGGTAGAGC ATCCCTACTG TAGCGAGTGC GCGGACAA  
 31681 CCGAGATCTGT GTTGTGCTGA GTGCTATGCC AAATGGAACG CCGGACGTAG TCATATTCTC  
 31741 TGAAGCAAAA CCAAGTGGCG CGGTGACAAA CAGATCTGCG TCTCGGTTCT CGGCTCTTAG  
 31801 ATGCTCTGT GTAGTAGTTG TAGTATATCC ACTCTCAAAA AGCATCTCGC GCGCCCTGAG  
 31861 CTTCCGGTTCT TATGTAAACT CCTTCATGCG CCGCTGCCCT GATACATCC ACCACCGCAG  
 31921 AATAAGCCAC ACCCAGCCAA CTTACACATT GCTTCTGCGA GCTTCTGCGA GCGAGGCGCG  
 31981 GAAGAGCTGG AAGAACCATG TTTTTTTTTT TATTCAAAA GATTATCCAA AACCTCAAAA  
 32041 TGAAGATCTA TTAAGTGAAC CGCTGCCCTT CCGGTGGCGT GGTCAAACTC TAGACGCAAA  
 32101 GAACAGATAA TGGCATTGTG AAGATTGGT ACAATGGCTT CAAAAGGCA AACCGCCCTC  
 32161 ACGTCCAAGT GGACGTAAAG GCTAAACCTT TCAGGGTGA TCTCTCTAT AAACATTTCA  
 32221 GCACCTTCAA CCATGCCCAA ATAATTCTCA TCGGCCACC TTCTCAATAT ATCTCTAAGC  
 32281 AAATCCCGAA TATTAAGTCC GGCCATTGTA AAACTCTGCT CCAGAGCGCC CTCCACCTTC  
 32341 AGCCTCAAGC AGCGAATCAT GATTGCAAAA ATTTAGGTTT CTCAAGACAT TGTATAAGT  
 32401 TCAAAAGCGG AACATTAAAC AAAATACCGG GATCCCGTAG GTCCCTTCGC AGGSCCAGCT  
 32461 GAACATAAAT GTGCAGGTCT GCACGGACCA GTCCCGGCCA TCCCGCCGCA GGAACCTTGA  
 32521 CAAAAGAACC CACACTGATT ATGACACGCA TACTCGGAGC TATGCTAAAC ACGGTAGGCC  
 32581 CGATGTAAAG TTTGTGTCAT GGGCGCGGAT ATAAAATGCA AGGTGCTGCT CAAAAATCA  
 32641 GGCAAGGCTC CGCGCAAAAA AGAAAGCACA TCGTAGTCAT GCTCATGAGC ATAAAGCGAG  
 32701 GTAAGCTCCG GAACCAACC AGAAAAGAGC ACCATTTTCT TCTCAACAT GTCTGCGGGT  
 32761 TTTGTCATAA ACACAAAAATA AAATAACAAA AAAACATTTA AACATTAGAA GCTGTCTTA  
 32821 CAACAGGAAA AACAAACCTT ATAAGCATAA GACGACTAC GGCCATGCG CGGTGACCGT  
 32881 AAAAAAACTG GTCAACCGTGA TTAAGAAAGCA CCACCGACAG CTCTCGGCTG ATGTCCGAG  
 32941 CATTAATGTA AGACTCGGTA AACACATCAT GTTGTATCAT CGGTGACTGC TAAAAGCGA  
 33001 CCGAATAGC CCGGGGGGAA ACATACCCGC AGGCGTAGAG ACACATTAAC AGCCCCCATTA  
 33061 GGAGGTATAA CAAAATTAAT AGGAGAGAAA AACACATAAA CACTCTAAAC ACCCTCTGCG  
 33121 CTAGGCAAAA TAGCACCTCT CCGCTCCAGA ACACATACA GCGCTTCAAC GCGCAGCGCT  
 33181 AACAGTCAGC CTTACCAATG AAAAAGAAAA CCTATTAAAA AAACACCCTT CGACACGGCA

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33241 CCAGCTCAAT CAGTCACAGT GTAAAAAAGG GCCAAGTGCA GAGCGAGTAT ATATAGGACT  
33301 AAAAAATGAC GTAACGGTGA AAGTCCACAA AAAACACCCA GAAACCCGCA CGCGAACCTA  
33361 CGCCCAAGAA CGAAAGCCAA AAAACCCACA ACTTCCTCAA ATCGTCACTT CCGTTTTCCC  
33421 ACGTTACGTA ACTTCCCAIT TTAAGAAAAC TACAATTCCC AACACATACA AGTTACTCCG  
33481 CCCTAAAACC TACGTCACCC GCCCCGTTCC CACGCCCGC GCCACGTCAC AAACCTCCACC  
33541 CCCTCATTAT CATATTGGCT TCAATCCAAA ATAAGGTATA TTATTGATGA TG

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kdl

FIGURE 22  
(SHEET 11)

11

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LOCUS            KD3                   34341 bp       DNA                   SYN                   06-FEB-1999  
 DEFINITION     KD3  
 ACCESSION     KD3  
 KEYWORDS  
 SOURCE        Unknown.  
 ORGANISM     Unknown  
               Unclassified.  
 REFERENCE     1 (bases 1 to 34341)  
 AUTHORS       Self  
 JOURNAL       Unpublished.  
 FEATURES  
   CDS  
     Location/Qualifiers  
       1..34341  
       /gene="KD3"  
       /product="KD3"

BASE COUNT       7951 a   9671 c   9464 g   7255 t  
 ORIGIN

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1  CATCATCAAT  AATATACCTT  ATTTTGGATT  GAAGCCAATA  TGATAATGAG  GGGGTGGAGT
61  TTGTGACGTG  GCGCGGGGCG  TGGGAACGGG  GCGGGTGACG  TAGTAGTGTG  GCGGAAGTGT
121  GATGTTGCAG  GTGTGGCGGA  ACACATGTAA  GCGAGCGGAT  TGGCAAAAGT  GACGTTTTTG
181  GTGTGGCGCG  GTGTACACAG  GAAGTGACAA  TTTTGGCGCG  GTTTTAGGCG  GATGTTGTAG
241  TAAATTTGGG  CGTAACCGAG  TAAGATTTGG  CCATTTTCGC  GGGAAAACGT  AATAAGAGGA
301  AGTGAAATCT  GAATAATTTT  GTGTTACTCA  TAGCGCGTAA  TATTTGCTCA  GGGCCGCGGG
361  GAGTTTGACC  GTTTACGTGG  AGACTCGCCC  AGGTGTTTTT  CTCAGGTGTT  TTCGCGTTTC
421  CGGGTCAAAG  TTGGCGTTTT  ATTATTATAG  TCAGCTGACG  TGTAGTGTAT  TTATACCCGG
481  TGAGTTCCTC  AAGAGGCCAC  TCTTGAGTGC  CAGCGAGTAG  AGTTTTCTCC  TCGAGCCCGC
541  TCCGACACCG  GGACTGAAAA  TGAGACATGA  GGTACTGGCT  GATAAFTCTC  CACCTGCTAG
601  CCATTTTGAA  CCACCTACCC  TTCAGCAACT  GTATGATTTA  GACGTGAGCG  CCCCAGGAAG
661  TCCCAACGAG  GAGGCGGTTT  CGCAGATTTT  TCCCGACTCT  GTAAATGTTG  CGGTGCAGGA
721  AGGGATTGAC  TTACTCACTT  TTCGCGCGCG  GCGCGGTTCT  CCGGAGCCGC  CTCACCTTTC
781  CCGCGAGCCC  GAGCAGCCGG  AGCAGAGAGC  CTTGGGTCCG  GTTTGCCACG  AGGCTGGCTT
841  TCCACCCGAG  GAGCAGCGAG  ATGAAGAGGG  TGAGGAGTTT  GTGTTAGATT  ATGTGGAGCA
901  CCGCGCGCAC  GGTTCGAGGT  CTTGTCAATTA  TCACCGGAGG  AATACGGGGG  ACCCAGATAT
961  TATGTGTTGG  CTTTGCTATA  TGAGGACCTG  TGGCATGTTT  GTCTACAGTA  AGTGAAATAT
1021  ATGGCGCAGT  GGTGATAGAG  TGGTGGGTTT  GGTGTGGTAA  TTTTMTTTCA  AATTTTITCA
1081  GTTTTGTGTT  TTAAGAATTT  TTGTATTGTT  ATTTTMTTAA  AAGGTCCTGT  GTCTGAACCT
1141  GAGCCTGAGC  CCGAGCCAGA  ACCGGAGCCT  GCAAGACCTA  CCCCCTGCTC  TAAATGGCGG
1201  CTGTCTATCC  TGAGACGCCC  GACATCACCT  GTGTCTAGAG  AATGCAATAG  TAGTACGGAT
1261  AGCTGTGACT  CCGGTCTCTC  TAACACACCT  CCTGAGATAA  ACCCGGTGGT  CCGCGTGTGC
1321  CCCATTAAAC  CAGTTGCGGT  GAGAGTTGGT  GGGCGTGACC  AGGCTGTGGA  ATGTATCGAG
1381  GACTTGTCTA  ACGAGCCTGG  GCAACCTTTG  GACTTGAGCT  GTAAACGCCC  CAGGCGATAA
1441  GGTGTAAACC  TGTGATTGCG  TGTGTGGTTA  ACGCCTTTGT  TTGCTGATGT  AGTTGATGTA
1501  AGTTTAAATA  AGGGTGAGAT  AATGTTTAA  TTGCATGCGC  TGTGTTAAAG  GGCGGGGCTT
1561  AAGAGGGTATA  TAATGCGCGC  TGGGCTAATC  TTGGTTTGGT  CTGACCTCAT  GCGCGCTTGG
1621  GAGTGTGTTG  AAGATTTTTT  TGCTGTGCGT  AACTTGCTGG  AACAGAGCTC  TAACAGTACC
1681  TCTTGGTTTT  GGAGGTTTCT  GTGGGGCTCA  TCCAGGCCAA  AGTTAGTCTG  CAGAGATTAC
1741  GAGGATTACA  AGTGGGAATT  TGAAGAGCTT  TTGAAATCCT  GTGTGAGCTT  GTTTGATTC
1801  TTGTAATCTG  GTCAACGAGC  GCTTTTCCAA  GAGAAGGTCA  TCAAGACTTT  GGATTTTCCC
1861  ACACCGGGGG  CGCGTGCGCC  TGCTGTTGCT  TTTTGTGAGT  TTATAAAGGA  TAAATGGAGC
1921  GAGAGAAACC  ATCTGAGCGG  GGGGTACCTG  CTGGATTTTC  TGGCCATGCA  TCTGTGGAGA
1981  CGCGTGTGGA  GACACAAGAA  TCGCCTGTGA  CTGTGTCTTT  CCGTCCGCCC  GGCATATAA
2041  CCGACCGAGG  AGCAGCAGCA  GCACAGGAGC  GAAGCCAGGC  GGCGCGCGCA  GAGCAGAGC
2101  CCATGTGAAC  CGAGAGCCGG  CCTGACCCTT  CGGGAATGAA  TGTGTGACAG  GTTGCTGAAC
2161  TGTATCCAGA  ACTGAGACGC  ATTTTGACAA  TTACAGAGGA  TGGCGCGGGG  CTAAGAGGGG
2221  TAAAGAGGGA  CGCGGGGGCT  TGTGAGGCTA  CAGAGGAGGC  TAGGAATCTA  GCTTTTAGCT
2281  TAATGACGAG  ACACGCTCCT  GAGTGATTTA  CTTTTCACCA  GATCAAGGAT  AATTGCGCTA
2341  ATGAGCTTGA  TCTGCTGGCG  CAGAAGTATT  CCATAGAGCA  GCTGACCACT  TACTGGCTGC
2401  AGCCAGGGGA  TGATTTTGAG  GAGGCTATTA  GGGTATATGC  AAAGGTGCGA  CTTAGGCCAG

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kd3

FIGURE 23  
 (SHEET 1)

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2461 ATTCCAAGTA CAAGATCAGC AAATCTGTAA ATATCAGGAA TTGTTGCTAC ATTTCTGGGA  
 2521 ACGGGGCCGA GGTGGAGATA ATAGGGTGGC CTTTATAGTG ATCATGATAA AGCATGATAA  
 2581 ATATGTGGCC GGGGGTGCTT GGCATGGACG GGGTGGTTAT TATGAATGTA AGGTTTACTG  
 2641 GCCCAAAATT TAGCGGTACG GTTTCCTGGG CCAATACCAA CTTATCTCTA CACGGTGTA  
 2701 GCTCTTATGG GTTTAACAAT ACCTGTGTGG AAGCCTGGAC CGATGTAAGG GTTCTGGGCT  
 2761 GTGCTTTTAA CTGCTGCTGG AAGGGGGTGG TGTTCTGCCC CAAAAGCAGG GCTTCAATTA  
 2821 AGAAATGGCT CTTTGAAGGG TGATCTTGGG GTATCTGTGC TGAGGGTAACT TCCAGGGTGC  
 2881 GCCACAATGT GGGCTCCGAC TGTGGTTGCT TCATGCTAGT GAAAAGCTGG GCTGTGATTA  
 2941 AGCATAAAGT GGTATGTGGC AACTGCGAGG ACAGGGCCTC TCAGATGCTG CACTGCTGGG  
 3001 ACGGCAACTG TCACCTGCTG AAGACCATTG ACGTAGCCAG CCACTCTCGC AAGGCCCTGG  
 3061 CAGTGTGTGA GCATAACATA CTGACCCGCT GTTCTTGTGA TTTGGGTAACT AAGGGTGGGG  
 3121 TGTCTCTACC TTACCAATGC AATTGAGTGC ACATAAAGT ATTGCTTGAG CCGAGAGACA  
 3181 TGTCCAAGGT GAACCTGAAC GGGGTGTTTG ACATGACCAT GAAGATCTGG AAGGTGCTGA  
 3241 GGTAACAGTA GACCCGACCC AGGTGCAGAC CCGTCGAGTG TGGCGGTAAA CATATTAGGA  
 3301 ACCAGCTGTG GATGCTGGAT GTGACGAGG AGCTGAGGCC CGATCACTTG GTGCTGGCT  
 3361 GCACCCGCGC TGAGTTTGGC TCTAGCGATG AAGATACAGA TTGAGGTACT GAAATGTGTG  
 3421 GCGCTGGCTT AAGGGTGGGA AAGAATATAT AAGGTGGGGG TCTTATGTAG TTTTGTATCT  
 3481 GTTTCGAGC AGCCGCGCCG GCCATGAGCA CCACTCGT TGATGGAAGC ATTGTGAGCT  
 3541 CATATTGAGC AAGCGCATG CCCCATGGG CCGGGGTGCG TCAGATGATG ATGGGCTCCA  
 3601 GCATGTGATG TCGCCCGCTC CTGCCCGCAA ACTCTACTAC CTTGACCTAC GAGACCGTGT  
 3661 GTTGAACGCC GTTGAGACT GCAGCCTCCG CGCCGCTTGC AGCCGCTGCA GCCACGCGCC  
 3721 GCGGATGTGT GACTGACTTT GCTTTCCTGA GCCCGCTTGC AAGCAGTGCA GCTTCCCGTT  
 3781 CATCCGCGCG GATGACAAG TTGACGGCTC TTTTGGCAGA ATTGGAITCT TGACCCGGGG  
 3841 AACTTAAATG CTTTCTCAG CAGCTGTGAG ATCTCGGCA GCAGTGTCTT GCCCTGAAGG  
 3901 CTTCTCTCCC TCCCAATGCG GTTAAAAACA TAAATAAAAA ACCAGACTGT TTTTGGATTT  
 3961 GGGTCAAGCA AGTGTCTTGC TGTCTTTATT TAGGGTGTTC GCGCCGCGGG GTGGCGCGGG  
 4021 ACCAGCGGTC TCGGTGTTG AGGGTCTGCT GTATTTTTTC CAGGACGTGG TAAAGGTGAC  
 4081 TCTGATGTTT CAGATACATG GGCATAAGCC CGTCTCTGTC GTGAGAGTAG CACCATGCA  
 4141 GAGCTTCATG CTGCGGGGTG GTGTTGTAGA TGATCCAGTC GTAGCAGGAG CGCTGGGGGT  
 4201 GGTGCCTAAA AATGTCTTTC AGTAGCAAGC TGATTGCGAG TGATTGCGAG GGGCAGGGCC  
 4261 TGTTTACAAA CGGTTAAAGC TGGGATGGTT GCATACGTTG GGATAAGAGA TGCATCTTGG  
 4321 ACTGTATTTT TAGGTTGGCT ATGTTCCGAC CATATCCCT CCGGGGATTC ATGTTGTGCA  
 4381 GAACACCCAG CACAGTGAT TCCGCTGACT TGGGAAATTT GTCATGTAGC TTAGAAGGAA  
 4441 ATGCGTGGAA GAACTTGGAG ACGCCCTTGT GACCTCCAAG ATTTTCCATG CATCTGCCA  
 4501 TAAATGATGGC AATGGGCCCA CGGGCGCGGG CCTGGCGGAA GATATTTCTG GGATCACTAA  
 4561 CGTCATAGTT GTGTTCCAGG ATGAGATCGT CATAGGCCAT TTTTACAAAG CGCGGGCGGA  
 4621 GGGTGCAGTA CTGCGGTATA ATGGTTCCAT CCGGCCCAGG GGCATAGTTA CCGTCAAGTA  
 4681 TTTGCATTTT CCACGCTTGG AGTTCAGATG GGGGGATCAT GTCTACCTGC GCGCGCATGA  
 4741 AGAAAACCGT TTCCGGGGTA GGGGAGATCA GCTGGGAAGA AAGCAGGTTC CTGAGCAGCT  
 4801 CGCACTTACC GCAGCGGGTG GGCCTGATAA TCACACTAT TACCGSGTGC AACTGTGAGT  
 4861 TAGAGAGACT GCAGCTGCGC TCATCCCTGA CGAGGGGGGG CACTTCCGTA AGCATGTCCC  
 4921 TGACTCGCAT GTTTTCCCTG ACCAAATCCG CCAGAAGGCG CTCGCGCGGTA AGGATAGCA  
 4981 GTTCTTGCAA GGAAGCAAG TTTTCAACG GTTGAGAGC TTCGCGCGTA GGCATGCTTT  
 5041 TGAGCGTTTG ACCAAGCAGT TCCAGCGCGT CCCACAGCTC GGTCAAGCTC TCTACGGCAT  
 5101 CTGATCCAG CATATCTCCT CGTTTCCGCG GTTGGGCGGG CTTTCCGCTG ACGGCASTAG  
 5161 TCGGTGCTCG TCCAGACGGG CAGGGTCAAT GTCTTTCAC GGGCGCAGGG TCTCTGTCAG  
 5221 CTGACTGTGG GTACCGGTGA AGGGGTGGCG TCCGGGCTCG CGCTGAGCCA GGGTGGGCT  
 5281 GAGGCTGTCT CTGCTGTGTC TGAAGCGCTG CCGGTTCTCG CCGTGTGCGT CCGCCAGGTA  
 5341 GCATTGACCC ATGTTGTGAT AGTCCAAGCC CTCGCGCGCG TGGCCCTTGT CCGCCAGCTT  
 5401 GCGCTTGGAG GAGGCGCGCG ACGAGGGGCA GTCCAGACTT TTGAGGGGCT AGAGCTTGGG  
 5461 CGCGAGAAAT ACCGATTCCG GGGAGTAGGC ATCCGCGCGC CAGGCCCCCG CAGCGGTCTC  
 5521 CATTTCACAG AGCCAGGTGA GCTCTGGCCG TTCGGGGTCA AAAACCAAGT TTTCCCCATG  
 5581 GTTTTGATG CTGTTCTTAC CTCTGGTTTC CATGAGCCGG TGTCACAGCT CGGTGACGAA  
 5641 AAGGCTGTCC GTGTCCCGGT ATACAGACTT GAGAGGCCGT TCCTCGAGCG GTGTTCCGCG  
 5701 CTCTCTCCCG TATAGAAACT CGGACCACTC TGAGACAAAG GCTCGGCTCC CAGCGCTCAG  
 5761 GAAGGAGGCT AAGTGGGAGG GGTAGCGGTC GTTGCTCACT AAGGGGTCCA CTCGCTCAG  
 5821 GTGTGAAGA CACATGTGCG CCTCTCGGC ATCAAGGAA GAGATTGGTT TGTAGGTGTA

FIGURE 23  
(SHEET 2)

5881 GGCCACCTGA CCGGGTGTTC CTGAAGGGGG GCTATAAAAG GGGGTGGGGG CGCGTTCGTG  
 5941 CTCACCTCTT TCCGCATCGC TGTCTGCGAG GGCCAGCTGT TGGGGTGGAT ACTCCCTCTG  
 6001 AAAAGCGGGC ATGACTTCTG CGCTAAGATT GTCAGTTTCC GTAGATTGAT AGGATTGAT  
 6061 ATTCACCTGG CCGCGGTGTA TGCTTTTGGG GTTGGCCGCA TCCATCTGAT CAGAAAAGAC  
 6121 AATCTTTTTT TTGTCAAGCT TGGTGGCAAA GCACCCGTAG AGGGCGTTGG ACAGCAACTT  
 6181 GGCGATGGAG CGCAGGGTTT GGTTTTTGTC GGATCGGGC CGCTCTCTGG CCGCGATGTT  
 6241 TAGCTGCAGC TATTGCGCGC CAACGCACCG CCATTTCGGGA AAGACGGTGG TGCGCTCGTC  
 6301 GGGCACCACG TGCACGCGCC AACCGCGGTT GTGCAGGGTG ACAAGGTCAA CGCTGGTGGC  
 6361 TACCTCTTCG CGTAGGCGCT CGTTGGTCCA CGAGAGGGCG CGCCCTCTGC CGGAGCAGAA  
 6421 TGGCGGTAGG GGGTCTAGCT CGCTCTCGTC CGGGGGGTCT GCGTCCACGG TAAAGACCCC  
 6481 GGGCAGCAGG CGCGGTGCGA AGTAGTCTAT CTTGCATCTT TGCAAGTCTG CACTCTCTGAG  
 6541 CCATGCGCGG CGCGCAAGCG CGCGCTCGTA TGGGTTAGT GGGGGACCCC ATGGCATGGG  
 6601 GTGGGTGAGC GCGAGGCGGT ACATGCCGCA AATGTCGTAA ACGTAGAGGG GCTCTCTGAG  
 6661 TATTCCAAAG TATGTAGGGT AGCATCTTCC ACCGCGGATG CTGGCGCGCA CGTATCGTAA  
 6721 TAGTTCTGTC GAGGGAGCGA GGAGGTGCGG ACCGAGGTTG CTACGGGCGG GCTGCTCTGC  
 6781 TCGGAAGACT ATCTGCCTGA AGATGGCATG TGAGTTGGAT GATATGGTTG GACGCTGGAA  
 6841 GACGTTGAAG CTGGCGTCTG TGAGACCTAC CGCGTCACGC ACGAAGGAGG CGTAGGAGTC  
 6901 GCGCAGCTTG TTGACCAAGT CGGCGGTGAC CTGCACGTCT AGGGCGGAGT AGTCCAGGGT  
 6961 TTTCTTGATG ATGTCACTAT TATCCTGTCC CTTTTTTTTT CACAGCTCGC GGTGAGGAC  
 7021 AAATCTCTCG CGGTCTTTCC AGTACTCTTG GATCGGAAAC CCGTCGGCCT CGGAACGGTA  
 7081 AGAGCGCTAG ATGTAGAAGT GGTTGACCGG CTGGTAGGGC CAGCATCCCT TTTCTACGGG  
 7141 TAGCGGTATG GCCTGCGCGG CTTTCCGGAG CGAGGTGTGG GTGAGGCGCA AGGTTGCCCT  
 7201 GACCATGACT TTGAGGTACT GGTATTGAA GTCAAGTGTG TCGCATCTCG CTGCTCCCA  
 7261 GAGCAAAAAG TCCGTGCGCT TTTTGGAAAG CGGATTGTGG AGGGCGGAAG TGACATCGTT  
 7321 GAAGAGTATC TTTCCGCGCG GAGGCATAAA GTTGCGGTG ATGCGGAAGG GTCCCGGCAC  
 7381 CTCGGAAACG TTGTTAAATTA CTTAGGCGG GAGCAGATC TCGTCAAAGC CGTTGATGTT  
 7441 TCGGCCCAAC ATGTAAAGTT CCAAGAAAGC CGGGA TGCCCT TTGATGGAAG CCAATTTTTT  
 7501 AAGTTCTCTG TAGGTGAGCT CTTAGGGGGA GCTGAGCCCG TGCTCTGAAA GGGCCAGATC  
 7561 TGCAAGATGA GGGTTGGAAG CGACGAATGA GCTCCACAGG TCACGGGCCA TTGACATTTG  
 7621 CAGGTGGTCG CGAAAGGTCC TAAACTGGCC ACCTATGGCC ATTTTTTCTG GGGTGAATGA  
 7681 GTAGAAGGTA AGCGGGTCTT GTTCCAGAGG GTCCCATCCA AGGTTGCGGG CTAGGTCTCG  
 7741 CGCGGCAGTC ACTAGAGGCT CATCTCCGCG GAACCTCATG ACCAGCATGA AGGGCACGAG  
 7801 CTGCTTCCCA AAGGCCCCCA TCCAAGTATA GGTCCTTACA TCGTAGGTGA CAAAGAGAGC  
 7861 CTGCGTGGCA GGATGCGAGC CGATCGGGA AAGTGGATC TCCCGCACCC AATTGGACAG  
 7921 GTTGCTATTG ATGTGGTGA AGTAGAAGTC CCGTGGCAGG GCGCAACACT GCTGCTGGCT  
 7981 TTTGTAAAAA CGTGCAGAGT ACTGGCAGCG GTGCACGGCG GTTACATCTT CCGTGGAGGT  
 8041 GAGCTGACGA CGCGSCACAA GGAAGCAGAG TGGGAATTG AGCCCTCTGC GTGCGCGGTT  
 8101 TGCTTGGTGG TCTTCTACTT CGGCTGCTTG CTCTGACCG CTGCGCTGCT CAGGGGAGAT  
 8161 TACGGTGGAT CGGACCAACA CGCCGCGCGA GCCCAAAGTC CAGATGTCCG CGCGCGCGCG  
 8221 TCGGAGCTTG ATGACAACAT CGCGCAGATG GAGCTGTCTC ATGTCTGGA AGTCCCGGCG  
 8281 CGTCAGGTCA GCGCGAGGCT CCGTGGAGTT TACCTCGCAT AGACGGGTCA GGGCGCGGCG  
 8341 TAGATCCAGG TGATACCTAA TTTCCAGGGG CTGGTTGGTG GCGGCGTGA TGGCTTGCAA  
 8401 GAGGCGCAT CCGCGCGGCG GCACTACGGT ACCGCGCGCG GGGCGGTGGG CCGCGGGGTT  
 8461 GTCTTTGGAT GATGCATCTA AAAGCGGTGA CGGCGCGGAG CCCCCGGAGG TAGGGGGGGG  
 8521 TCCGGACCCG CCGGAGGAGG CAGCAGGGCG ACGTCCGGCG CGCGCGCGGG GCGATCTGG  
 8581 TGCTGCGCGC GTAGGTTGCT GCGGAAGCGC ACGACGCGCG GGTGATCTCT CTGAATCTGG  
 8641 CGCTCTCGCG TGAAGACGAC GGGCCCGGTG AGCTTGAGCC TGAAAGAGAG TGACACAGAA  
 8701 TCAATTTCCG TGTCGTTGAC GGGCGGCTGG CGCAAAATCT CTTGCAAGTC TCGTAGTTG  
 8761 TTTGATAGG CGATCTCGGC CATGAACTGC TCGATCTCTT TCGATAGTCA CGCCCTTTC  
 8821 CGCGCTCGCT CCACGGTGGC GGGGAGGTGG TTTGAAATGC GGGCCATGAG CTGCGAGAGG  
 8881 CGGTTGAGGC CTCCCTCGTT CCAGACGCGG CTTGAGTCA CGCCCCCTTC GGCATCGCGG  
 8941 CGCGCGATGA CCACCTCGCG GAGATTGAGC TCCACGTGCC GGGCGAAGAC GGGGTAGTTT  
 9001 GCGAGGCGCT GAAAGAGGTA GTTGAAGGTT GTTTCGCAC GAAAGAGTAC GAGGAGTAC  
 9061 ATAAACCAAG GTCCGCAAGT GGAATTGTTG ATATCCCCCA AGGCTCTCAAG GCGCTCCATG  
 9121 GCTCTGTAGA AGTCCACGCG CAAGTTGAAA AACTGGGAGT TGCGCGCGCA CACGGTTAAC  
 9181 TCTCTCTCCA GAAGACGGAT GAGCTCGGCG ACAGTGTGCG GCACCTCGCG CTCAGGGCT  
 9241 ACAGGGGCGT CTTCTTCTTC TTCAATCTCC TCTTCATAAA GGGCTCTCCC TTCTCTTCT

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9301 TCTGGCGCGG GTGGGGGAGG GGGGACACGG CGGCGACGAC GGGCGCACGG GAGCGGGTCG  
 9361 ACAAGCGCCT GCATCATCTC CCGCGGGCGA CGGCGCATGG TCTCGGGTAC GGGCGGGCGG  
 9421 TTCTCGCGGG GGGCGAGTTG GAAGACGCGG CCGGTCATGT CCGGGTATG GGTGTGGCGG  
 9481 GGGCTGCCAT CGGCGAGGGA TACGGCGCTA ACGATGCATC TCAACAATGT TTGTTAGTGT  
 9541 ACTCCGCCGC CGAGGGACCT GAGCGAGTCC GCATCGACCA GATCGGAAAA CCTCTCGAGA  
 9601 AAGGCGTCTA ACCAGTCACA GTGCGAAGGT AGGCTGAGCA CCGTGGCGCG CGGCGAGCGG  
 9661 CGGCGGTCGG GGTGTCTTCT GCGGAGGTG CTGCTGATGA TGTAAATAAA GTAGGCGGTC  
 9721 TTGAGACGGC GGATGCTCGA CAGAAGCACC ATGCTCTTGG GTCCGGCGCT CTGAATGCGC  
 9781 AGGCGGTCGG CCATGCCCCA GGTCTTGTTT TGACATCGGC GCAGGTCCTT GTAGTAGTGT  
 9841 TGCATGAGCC TTCTACCGG CACTCTCTCT TCTCTCTCTC CTCTGCTGCG ATCTCTTGCA  
 9901 TCTATCGCTG CGGCGGCGGC GGAAGTTTGG CGTAGGTTGG GCGCTCTTCC TCCCATGCGT  
 9961 GTGACCCCGA AGCCCTCAT CTGCTGAAGC AGGGCTAGGT CGGCGACAAC GCCTCGGCT  
 10021 AATATGGCCT GCTGCACCTG CTGAGGGTGA GACTGGAAGT CATCCATGTC CACAAAGCGG  
 10081 TGGTATGCGC CCGTGTGAT GGTGTAAGTG CAGTTGGCCA TAACGAGCCA GTTAACCGTC  
 10141 TGTGTACCGG CTTGCGAGAG CTCGGTGTAC CTGAGACGCG AGTAAGCCCT CGAGTCAAA  
 10201 ACGTAGTCGT TGCAGTCCG CACCAGGTAC TGGTATCCCA CCAAAAAGTG CGGCGGCGG  
 10261 TGGCGGTAGA GGGGCCAGAG TAGGGTGGCC GGGGCTCGG GGGCGAGATC TTCCAACATA  
 10321 AGCGCATGAT ATCCGTAGAT GTACCTGGAC ATCCAGTGA TGCCGGCGCG GTGGTGGAG  
 10381 GCGCGCGGAA AGTCGCGGAC GCGGTTCCAG ATGTTGCGCA CGGCGACAAA GTGCTCATG  
 10441 GTCGCGACGC TCTGSCCGGT CAGCGCGCGC CAATCGTTGA CGCTCTAGCG TGCAAAAGGA  
 10501 GAGCTGTAA GCGGCACTC TTCCGTGGTC TGGTGATAA ATTCCGAAGG GTACTATGGC  
 10561 GGAACGACCG GGTTGAGGCC CGGTATCCGG CGGTCCGCGC TGATCCATGC GGTATCCGCC  
 10621 CGCGTGTGCA ACCCAGGTGT GCGACGTGAC ACAACGGGGG AGTGCTCCTT TTGGCTTCTT  
 10681 TCCAGGCGCG GGGCTGCTG CTGATGCTTT TTGGGCGACT GCGCCGCGCT GCGGTAAGCG  
 10741 GTTAGGCTGG AAGCGAAAG CATTAAAGTG CTGCTCCCT GTAGCGGAGG GGTATTTTTC  
 10801 CAAGGGTTGA GTGCGGGGAC CCGCGTTGCG AGTCTCGGAC GCGCGGAGAT TGCCACAAAT  
 10861 GGGGTTGGCC TCCCGGTGAT GCAAGACCCC GCTTGCAAAAT TCCTCCGGA ACAGGAGCGA  
 10921 GCGCTCTTTT TGCTTTTCCC AGATGCATCC GGTGCTCGGG CAGATGCGCC CCCCTCTCTA  
 10981 GCGAGCGGCA GAGCAAGAGC AGCGGACGAC ATGCAAGGCA CCCTCCCTC CTCTACCGC  
 11041 GTCAGGAGGG GCGACATCCG CGGTTGACGC GGCAGCAGAT GGTGATTAC AACCCCGCG  
 11101 GCGCGCGGCC GCGCACTACC TGGACTTGA GAGGGGCGAG GGCCTGCGC GGTCTAGAGC  
 11161 GCGCTCTCTT GAGCGGTACC CAAGGGTGCA GCTGAAGCGT GATACGCGTG AGGCGTACGT  
 11221 GCGCGGCGAG AACTGTCTTC GCGACGCGCA GGGAGAGGAG CCGAGGAGA TGCGGGATCG  
 11281 AAGTCTCCAC GAGGGGCGCG AGCTGCGGCA TGCCCTGAAT CGCGAGCGGT TGCTGCGGCA  
 11341 GAGGAGCTTT GAGCCGACG CCGGAACCGG GATTAGTCCC GCGCGCGCT ACGTGGCGGC  
 11401 CGCGACCTGT GTAACGCAT ACGAGCAGAC GGTGAACGAG GAGATTAACT TTCAAAAAG  
 11461 CTTTAAACAC CAGCTCGTA CCGTTGTGGC GCGCGAGGAG GTGGCTATAG CAGTATGCA  
 11521 TCTGTGGAGC TTTGTAAGCG CGCTGGAGCA AAACCCAAT AGCAAGCCGA CTGTGCGCA  
 11581 GCTGTTCTTT ATAGTGCAGC ACAGCAGGA CAACAGGCA TTCAGGATG CCGTGTAAA  
 11641 CATAGTAGAG CCCAGGGGCC GCTGGCTGCT CGATTGATA AACATCTGT AGAGCATAGT  
 11701 GGTGAGAGG CGAGCCTTGA GCTTGGCTGA CAAGGTGGCC GGCATCAACT ATTCATGCT  
 11761 TAGCCTGGCC AAGTTTACG CCGCAAGAT ATACCATACC CCTTAGTTTC CATATGACAA  
 11821 GAGGTTAAAG ATCGAGGGGT TCTCATGTG CATGGCGCTG AAGGTGCTTA TCTTAGGCA  
 11881 CGACTCTGGC GTTTATCGCA ACGAGCGCAT CCACAAAGCC GTGAGCGGTA GCGGGCGCG  
 11941 CGAGCTCAGC GACCGGAGC CTGTGACAG CCTGCAAGG CGCCTGCTGA GCGAGCGGCG  
 12001 CGCGGATAGA GAGGCGAGT CTAATTGTA GCGCGGCGCT GACCTGCGCT GGGCCCAAG  
 12061 CCGAGCGGCC CTGGAAGCAG CTGGGGCGCG CCGGTGGCAC CGGCTGGCAC CGCGCGCGC  
 12121 TGGCAAGCTC GCGGCGGTGG AGGAATATGA CGAGAGCAGT GAGTACGAGC CAGAGAGCGG  
 12181 CGAGTACTAA CGGCTGATGT TTCTGATCAG ATGATGCAAG ACAGCAAGGA CCGCGCGGTG  
 12241 CGGGCGGCGC TGCAGAGCCA GCGGTCGCGC CTAACTCCA CGGAGCAGTG GCGCGAGGTC  
 12301 ATGAGACGCA TCAATGCGCT GACTGCGCGC AATCTGACG CGTTCCGCGA CGAGCGCAG  
 12361 GCGAACCGGC TCTCCGAAT TCTGGAAGCG GTGGTCCCGG CCGCGCAAA CCGCACGAC  
 12421 GAGAGGTTGC TGGCGATCGT AAACCGGCTG GCGCAAAACA GGGCATCTG CCGGACGAG  
 12481 CGCGGCGTGG TCTACGAGC GCTGCTTCA GCGGTGGCTG GTTACAAAG CCGCAACGTG  
 12541 CAGACCAACC TGACCCGCT GGTGGGGGAT GTGCGCGCA CGGTGCGCA CGGTGAGCG  
 12601 GCGCAGCAGC AGGCGAACT GGGCTCCATG GTTGCACTAA ACCTCTCTT GAGTACAGC  
 12661 CCGCGCAACG TCCCGCGGGG ACAGAGGAGC TACACCAACT TTGTGAGCGC ACTGCGGCTA

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FIGURE 23  
(SHEET 4)

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12721 ATGGTGACTG AGACACCGCA AAGTGAGGTG TACCAGTCTG GGCCAGACTA TTTTTCACG  
 12781 ACCAGTAGAC AAGGCGTGCA GACCGTAAAC CTGAGCCAGG CTTTCAAAAA CTTGACAGGG  
 12841 CTGTGGGGGG TGCGGCGTCC GACGAGCGAC CACGAGCGAC TGTCTAGCTT GCTGACGCTT  
 12901 AACTCGCGCC TGTGTGCTCT GCTAATAGCG CCGTTCACGG ACAGTGGCAG CGTGTCGCGG  
 12961 GACACATACC TAGGTCACCT GCTGACACTG TACCGCGAGG CCATAGTGTA GGCGCATGTG  
 13021 GACGACGATA CTTTCCAGGA GATTACAGT GTACCGCGCG CGCTGGGGCA GGAGGACACG  
 13081 GCGAGCTGAG AGGCAACCGT AACTACCTCT CTGACCAACC GTGCGCAGAA GATCCCTCTG  
 13141 TTGCACAGTT TAAACACGGA GGAGGAGCGG ATTTTGGCGT ACCTGTCAGC GAGCGTGAGC  
 13201 CTTAACCTGA TGCGGACGGG GGTAAACGCC AGCGTGGCGC TGACATGTAC CGCGCGAAC  
 13261 ATGAGACCGG GCATGTATGC CTCAAACCGG CCGTTTATCA ACCGCTTAAT GGACTACTTG  
 13321 CATCGCGCGG CGCGCGTGAA CCGCGAGTAT TTACCAATG CCATCTCTGG CCGCGACTTG  
 13381 CTACCGCGCC CTGGTTTCTA CACCGGGGGA TTGAGGTGTC CCGAGGGTAA CGATGGATTG  
 13441 CTTCTGGGAG ACATAGACGA CAGCGTGTGT TCCCGGCAAC CGCAGACCTG GTAGAGTTTG  
 13501 CAACGACGGG AGCAGGCGGA GGCGGCGCTG CGAAAGGAAA GCTTCCGACG GCCAAGCAGC  
 13561 TTGTCCGATC TAGGCGCTGC GGCGCGCGGG TCAGATGCTA GTAGCCCAAT TCCAAGCTTG  
 13621 ATGGGGTCTC TTACCGAGCA TCGCACCAAC CGCGCGCGCC TGCTGGGCGA GGAGGAGTAC  
 13681 CTAAACAACCT CGCTGTGCTA CGCGCAGCGC GAAAAAACC TGCTCCGCGC ATTTCCCAAC  
 13741 AAGGGGATAG AGAGCCTAGT GGAACAAGAT AGTAGATGGA AGACGTACGC GCAGGACGAC  
 13801 ACGGAGCTGC CAGGCGCGCG CGCGCCCAAC CGTCTCAAA GGCAACGACG CTACGGGGGT  
 13861 CTGCTGTGGG AGGACGATGA CTCGGCAGAC GACAGCAGCG TCTGTGATTG GGGAGGGAGT  
 13921 GCAACCCGAT TTGCGCACTT TCGCCCAAG TCGGGAGAAA TGTTTAAAA AAAAAAAGC  
 13981 ATGATGCAAA ATAAAAAACC CACCAAGGCC ATGGCACCGA GCCTTGGTTT TCTGTATTTC  
 14041 CCGCTTAGTAT CGCGCGCGCG CGGATGTATG AGGAAGGTCC TCCTCCCTCC TACGAGAGTG  
 14101 TGCTGAGCGC GCGCGCAAGT GCGCGCGCGC TGCGTTCTCC CTTGATGTCT CCGCTGAGCC  
 14161 CGCGTTTGTG GCCTTCGCGG TACCTGCGCG CTACCGGGGG GAGAAACAGC ATCCGTATTCT  
 14221 CTGAGTTGAG ACCCTTATTC GACACCAACC GTGTGTACTT GTGTGACTAC AGTGTCAACG  
 14281 ATGTGCAATC CCGTGAACAT CAGAACGACC ACAGCAACCT TCTGACCAAG GTCAATCAAA  
 14341 ACAATGACGA CAGCCCGGGG GAGGCAGACA CACAGCAAT CAATCTTGAC GACCGGTGCG  
 14401 ACTGGGGCGG CGACTGTGAA ACCATCTGTC ATACCAACAT GCCTAATGTG AACGAGTTCA  
 14461 TGTTTACCAA TAAAGTTTAA GCGCGGGTGA TGGTGTGCGG CTGCGCTACT AAGGACAATC  
 14521 AGGTGGAGCT GAAATACGAG TGGGTGGAGT TCACTGCTGC CGAGGGCAAC TACTCCGAGA  
 14581 CCATGACCAT AGACCTTATG AACAACGCGA TCGTGGAGCA CTACTTGAAA TGGGGAGAGC  
 14641 AGAACGGGGT TCTGAAAAGC GACATCGGGG TAAAGTTTGA CACCGCAAC TTGAGCTGG  
 14701 GGTGTGACCC CGTCACTGGT CTGTGCTATG CTGGGGTATA TACAACGAA GCCTTCCATC  
 14761 CAGACATCAT TTTGCTGCCA GGAATGCGGG TGGAAGTTCAC CCACAGCGCG CTGAGCAACT  
 14821 TGTGTGGCAT CCGCAAGCGG CAACCTTCC AGGAGGGGCT TAGGATCAC TACATGATC  
 14881 TGGAGGGTGG TAACTATCCC GCACTGTGAG ATGTGGAGCG CTACAGGCGC AGCTTGAAG  
 14941 ATGACACGGA ACAGGGCGGG GGTGGCGCAG CGCGCAGCAA CAGCATGAGC AGCGCGCGG  
 15001 AAGAGAACTC CAACGCGGCA GCGCGGCGAA TGCAGCCGCT GGAGGATCAT AACGATCATG  
 15061 CCAATGCGGG CGACACCTTT GCGCAACGGG CTGAGGAGAA GCGCGCTGAG CGCGAGCAG  
 15121 CGGCGGAAGC TGCGCGCCCC GCTGCGCAAC CCGAGGTGGA GAAGCTTCAG AAGAACCGG  
 15181 TGCTCAAACC CCGTACAGAG GACAGCAAGA AACGCAAGTTA CAACCTAATA AGCAATGACA  
 15241 GCACCTTCAAC CCAATACCGC AGCTGGTACC TTGCATACAA CTACGGCGAC CCGTACAGCG  
 15301 GAATTCGCTC ATGGACCCCT GTTTGCACTC CTGACGTAAAC CCGTGGCTCG GAGCAGATCT  
 15361 ACTGCTCGTT GCGAGACATG ATGCAAGACC CCGTGAACCT CCGTCCACG CCGCAGATCA  
 15421 GCAACTTTCC GGTGGTGGCG GCGGAGCTGT TGCCGTGCA CTCCAGAGCG TTCTACAAG  
 15481 ACCAGGCGGT CTACTCCCAA AGTTACCGC AGTTTACCTC TTGACGCCAC GTGTGTAATC  
 15541 GCTTTCCCGA GAACAGATT TTGGCGCGCG CGCCAGCCCC CACCATCACC ACCGTGAGT  
 15601 AAAACGTTCC TGCTCTACA GATCACGGAG GCGTACGCGT CGCTACGAGG ATCGGAGGAG  
 15661 TCGAGCGAGT GACCATTAAT GACGCGAGG GCGCGACCTG CCGCTACGTT TACAAGGCC  
 15721 TGGGCATAGT TCGCGCGCGC GTCTATGGA GCGCACTTTT TTGAGCAAGC GTGTCATCT  
 15781 TTATATGCGC CAGCAATAAC ACAGGCTGGG GCTTGCCTCT CCGAAGCAAG AGTGTGGCG  
 15841 GGGCCAGAAA GCGCTCCGAC CAACACCCAG TCGCGGTGCG CGGCGACTAC CGCGCGCCT  
 15901 GGGCGCGCGA CAACCGCGCG CCGCATGGCG GCACCAACGT CGATGACCGC ATGCAACGCG  
 15961 TGCTGGAGGA GCGCGCAAC TACAACGCAA AGTGTCCACA GTGCGACCGG GCGCGAGCG  
 16021 CATTTAGAC CCGTGGTGGC GGAGCCGCGC GCTATGCTAA AATGAAGAGA CCGCGAGGCG  
 16081 GGTATGACCG TCGCCACCGC CGCGGACCG GCGCGCGCG CCAACGCGCG CCGCGCGGCC

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16141 TGCTTAACCG CGCACGTCGC ACCGCGCGAC GGGCGGCCAT GCGGGCCGCT CGAAGGCTGG
16201 CCGCGGGTAT TGTCACCTGT CCCCACAGGT CCAGCGGACG AGCGGGCCGGT CGCAGCAGCG
16261 CGCGCAATTAG TGCTATGACT CAGGGTCGCA GGGGCAACGT GTATTGGGTG CGCGACTCGG
16321 TTAGCGGGCT CGCGGTGCC CGTGCGCACCC GCGCCCGCG CGACTAGATT CGAAGAAAAA
16381 ACTACTTAGA CTCGTACTGT TGTATGTATC GACCGCGCG GCGCGCGAAC GAAGCTATTG
16441 CCAAGCGCAA AATCAAGAA GAGATGCTCC AGGTATCTGC GCCGGAGATC TATGGCCCCC
16501 CGAAGAAGGA AGACAGGAT TACAAGCCCC GAAAGCTAAA GCGGGTCAAA AAGAAGAAAA
16561 AAGATGATGA TGTGAACCT GACGACGAGG TGGAACTGCT GCACGCTACC CGCCGCCAGC
16621 GACGGGTACA GTGGAAGGT CGACGCGTAA AACGTGTTTT GCGACCCGGG ACCACCGTAG
16681 TCCTTACGCC CGGTGAGCGC TCCACCCGCA CCTACAAGCG CGTGTATGAT GAGGTGTACG
16741 GCGACGAGGA CCTGCTTGAG CAGGCCAAAG AGCGCTCGG GAGTGTITGCC TACGGAAGAG
16801 GGCATAAGGA CATGCTGGCG TTGCGCGTGG ACGAGGGCAA CCCAACACCT AGCCTAAAGC
16861 CCGTACACCT GCAGCAGGTG CTCGCCGCGC TTGCACCGCT TGCACCGCTA GCGGCGCTAA
16921 AGCGCGAGTC TGTGTACTTG GCACCCACCG TGCAGCTGAT GGTATCCCAAG CGCCACGCAAG
16981 TGAAGATGTT CTTGGAAGAA ATGACCGTGG AACCTGGGCT GGAGCCCCGG CTCGCGGTGC
17041 GGCCAATCAA GCAGGTGGCG CGGGACGTGG CGGTGCAGAC CGTGGACGTT CAGATACCCA
17101 CTACCACTAG CACCAGTATT GCCACCGCCA CAGAGGGCAT GGAGACACAA AGCTCCCGGG
17161 TTGCTCAGC GGTGGCGGAT GCGCGGTGTC AGGCGGTTCG TGGCGCCCGC TCAGAAGCTC
17221 CTACGAGGTT GCAAAAGGAC CGTGGATATT TTGCGTITTT AGCCCCCGGG CGCGCGCGCG
17281 GTTCGAGGAA GTACGGCGCC GCCAGCGGCG TACTGCCGGA ATATGCCCTA CATCTTCCA
17341 TTGCGCTTAC CCCCCTATC CGTGGCTACA CCTACCGGCG CAGAAGAGCA GCAACTACCC
17401 GACCGCGAAC CACCACTGGA ACCCGCGCGC GCGGTGCGCC GCGCGAGGCT CCACAGCGCT
17461 CGATTTCGGT CGCAGGGGTG GCTCGCGAAG GAGCGCAGAC CCTGTGTGCT CCAACAGGCT
17521 GCTACACCCC CAGCATCGTT TAAAGCCGTT TCTTTGGTGT TCTTGTAGAT AGGACCTTCA
17581 CCGTCGCGCT CGGTTTCCCG GTGCGCGGAT TCGCAGGAAG AATGCACGGT AGGAGGGGCA
17641 TGGCGCGCCA CGGCTGACG GCGCGCATCG TCGGTGCGCA CCACCGCGGG CGCGCGCGGT
17701 GCACCGCTGC CATGCGCGCG GGTATCTCTC CCTCCTTAT TCCACTGATC CGCGCGGCA
17761 TTGCGCCGCT GCCCGGAATT GCATCGTGG CCTTGCAGGC GCAGAGACAC GTATTAAAAA
17821 CAAGTGTGAT GTGGAAGAAAT GAAATAAATA AGTCTGGACT CTCACGCTCG CTGTGCTCTG
17881 TAACTATTTT GTAGAATGGA AGACATCAAC TTGCGTCTC TGGCCCGCGC ACACGGCTCG
17941 CGCCCGTTCA TGGGAAACTG GCAAGATATC GGCACACGCA ATATGAGCGG TGGCGCTTC
18001 AGCTGGGGCT CGCTGTGGAG CGGCATTAAA AATTTGCGTT CCACCGTTAA GAACTATGGC
18061 AGCAAGGCGT GGAACAGCAG CACAGGCGAG ATGCTGAGGG ATAAGTTGAA AGAGCAAAAT
18121 TTCCAACAAA AGGTGGTAGA TGGCTGGGCC TCTGGCATTA GCGGGGTGGT GGACCTGGCC
18181 AAGCAGGCGAG TGCAAAATAA GATTAAACAGT AAGCTTGATC CCCGCCCTCC CTGAGAGGAG
18241 CCTCCACCGG CGGTGGAGAC AGTGTCTCCA GAGGGGCGTG GCGAAGAAAGC CGTACGCCCC
18301 GACAGGGAAG AAATCTGTGT GACGCAATAA GACGAGCCTC CCTGTACGA GAGGAGCACT
18361 AAGCAAGGCC TGCCCAACCA CGTCTCCATC GCGCCATGCG CTACCGAGAT CTGCGGCCAG
18421 CACACACCCG TAACTGTGGA CTTGCTGCC CCCGCCGACA CCACAGCAAC ACCTGTGCTG
18481 CCAGGCGCGA CGCGCTTGT TGTAAACCGT CTAGCCGCG CGTCCCTGCG CTGCGCGCGC
18541 AGCGGTGCGG GATCGTTGCG GCCGTGAGC AGTGCAACT GGCAGAGCAC ACTGAACAGC
18601 ATCGTGGGTC TGGGGGTGCA ATCCCTGAAG CCGCGACGAT GCTTCTGAAT AGCTAACGTC
18661 TCGTATGTGT GTCATGTATG GTCCTATGTC GCGCCAGAG GAGCTCGCGC GCGCGCGCGC
18721 GCGCGCTTTC CAGATGGGCT ACCCTTCTGA TGTATCCGCA GTGGTCTTAC ATGCACATCT
18781 CGGGCCAGGA CGCTCGGAG TACCTGAGCC CGGGCTGTGT CGAGTTTGGC ACAGCACGCG
18841 AGACGTACTT CAGCCTGAAT AACAAAGTTA GAAACCCAC GGTGGCGGCT ACGACGAGAG
18901 TGACCACAGA CCGTCCACG CGTTTACGCG TGTGGTTCAT CCTGTGTGAC CTGAGGATA
18961 CTCGTGATCT GTACAAGGCG CGGTTACACC TAGCTGTGGG TGATAAACGT GTGCTGGACA
19021 TGGCTTCCAC GTACTTTGAC ATCCGCGGCG TGTGGGACAG GGGCCCTAGC TTTAAGCCCT
19081 ACTCTGGCAC TGCTTACAA CCGCTGGCTC CCAAGGGTGC CCCAAATCCT TGGCAATGGG
19141 ATGAAGCTGC TACTGTCTTT GAAATTAACC GAACTAGAGA GACGATGAC AACGAAGACG
19201 AAGTAGAGGA GCAAGCTGAG CAGCAAAAAA CTCACGTATT TGGCGAGGCG CTTTATTCTG
19261 GTATAAATAT TACAAGGAG GGTATTCAAA TAGGTGTGCA AGGTCAACAA CTTAAATATG
19321 CCGATAAANA ATTTCAACCT GAACCTCAAA TAGGAGAATG TCAGTGGTAT GAAACTGAAA
19381 TTAATCATCG AGCTGGGAGA GTCCCTAAAA AAGTAAACCC AATGAACCCA TGTTACGGTT
19441 CATATGCAAA ACCCACAAT GAAATGGAG GCAAGGCAT TCTTGTAAAG CAACAAATG
19501 GAAAGCTAGA AAGTCAAGTG GAAATGCAAT TTTTCTCAAC TACTGAGGCG ACCCGAGGCA

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5c/6c

19561 ATGGTGATAA CTTGACTCCT AAAGTGGTAT TGTACAGTGA AGATGTAGAT ATAGAAACCC  
 19621 CAGACACTCA TATTTCCTAC ATGCCCACTA TTAAGGAAGG TAACTCACGA GAACATAAGG  
 19681 GCCACAATC TATGCCCAAC AGGCCTAATT ACATTGCTTT TAGGGACAAT TTTATTGGTC  
 19741 TAATGTATTA CAACAGCACG GGTAATATGG GTGTCTCGGC GGGCCAAGCA TCGCAGTTGA  
 19801 ATGCTGTTGT AGATTTCGAA GACAGAARCA CAGAGCTTTC ATACCACTGT TTGCTTGATT  
 19861 CCATTGGTGA TAGAACCAGG TACTTTTCTA TGTGGAATCA GGCTGTGTAC AGCTATGATT  
 19921 CAGATGTTAG AATTATTGAA AATCATGGAA CTGAAGATGA ACTTCCAATC TACTGCTTTC  
 19981 CACTGGGAGG TGTGATTAAT ACAGAGACTC TTACCAAGGT AAAACCTTAA ACAGGTCAGG  
 20041 AAAATGGATG GGA AAAAGAT GCTACAGAAT TTTACAGATA TTTCAACGTA AGATTGGAA  
 20101 ATAATTTTGC CATGGAATC AATCTAAATG CCAACCTGTG GAGAAATTTT CTGTACTCCA  
 20161 ACATAGCGCT GTATTTCGCC GACAAGCTAA AGTACAGTCC TTCCAACGTA TTTCACTTTC  
 20221 ATAAACCAA CAACCTACGAC TACATGAACA AGCGAGTGGT GGCTCCCGGG TTAGTGGACT  
 20281 GCTCATTTAA CCTTGGAGCA CGCTGGTCCC TTGACTATAT GGACAACTCT AACCCATTTA  
 20341 ACCACACCG CAATGCTGGC CTGCGCTACC GCTCAATGTT GCTGGGCAAT GGTCGCTATG  
 20401 TGCCCTTCCA CATCCAGGTG CTTCAGAAAT TTTTGCCAT TAAAAACCTC TTCTCTCTGC  
 20461 GCGGTCGATA CACCTACGAG TGGAACTTCA GGAAGGATGT TAACTAGTT CTGACAGGCT  
 20521 CCCTAGGAAA TGACCTAAGG GTTGACGGAG CCAGCATTAAG GTTTGATAGT ATTTGCTTTT  
 20581 AGGCCACCTT CTTCGCCATG GCCCACAACA CCGCTCCACG GCTTGAGGCC ATGCTTAGAA  
 20641 ACAGACCAA CGACCAGTCC TTAAAGCACT ATCTCTCCGC CCGCAACTG GCTTACCOCTA  
 20701 TACCCGCCAA CGCTACCAAC GTGCCCATAT CCATCCGCCCT CCGCAACTG GCGGCTTTCC  
 20761 GCGGCTGGGC CTTACGCGGC CTTAAGACTA AGGAAACCCC ATCACTGGGC TCGGGCTACG  
 20821 ACCCTTATTA CACTACTCTT GGCTCTATAC CTTACCTAGA TGGAACTTTT TACCTCAACC  
 20881 ACACCTTTAA GAAGGTGGCC ATTACTTTG ACTCTTCTGT CAGCTGGCTG GGCATGAGC  
 20941 GCGTCTTAC CCCCACGAG TTTGAAATTA AGCGCTCAAT TGAGCGGGAG GTGTACACAG  
 21001 TTGCCAGTG TAACATGACC AAAGACTGGT TCCTGGTACA AATGTAGCT ACTACACAA  
 21061 TTGGGTACCA GGGCTTCTAT ATCCCAAGGA GCTACAGATC CGCATGTATC TCTCTCTTTA  
 21121 GAACTTTCCA GCCCATGAGC CGTCAGGTGG TGGATGATAC TAAATACAGT GACTACCAAC  
 21181 AGGTGGGCAT CCTACACCAA CACAACAAT CTGGAATTTG TGGCTATGCT GACCCACCAA  
 21241 TGCGCGAAGG ACAGGCCCTAC CCGTCTAATC TCCTTATCC GCTTATAGCG AAGACCGCAG  
 21301 TTACACGAT TACCCAGAAA AAGTTTCTTT GCGATGCGAC CCGTATGCGAG ATCCCATCTT  
 21361 CCACTAATCT TATGTCATG GCGGCACTCA CAGACTGGG CCAAAACCTT CTTCTACGCCA  
 21421 ACTCGGCCCA CGCGCTAGAC ATGACTTTTG AGGTGGATCC CATGAGCGAG CCGACCTTTC  
 21481 TTTATGTTTT GTTTGAAGTC TTTGAGTGGT TCGGTGTGCA CCGGCGGCAC CCGCGCGTCA  
 21541 TGGAAACCGT GTACCTGCGC ACSCCTTTCT CGGCGCGCAA CGCCACAACA TAAAGAGACA  
 21601 AGCAACATCA ACAACAGCTG CCGCATGGG CTCCAGTGAG CAGGAACCTA AAGCCATTTT  
 21661 CAAAGATCTT GGTGTGGGCG CATATTTTTT GGGCACCTAT GACAAGGCTT TTCCAGGCTT  
 21721 TGTTTCTCCA CACAAGCTCG CCTGGGOCAT AGTCAATACG CCGCGTGGC AGACTGGGGG  
 21781 CGTACACTGG ATGGCCTTTG CCTGGAACCC GACTCAAAA ACATGCTACC TCTTTGAGCC  
 21841 CTTGGCTTT TCTGACCCAG GACTCAAGCA GGTTTACGAG TTTGAGTACG AGTCACTCCT  
 21901 GCGCGTATGC GCCATTGCTT CTTCGCCCGA CCGCTGTATA ACGCTGGAAT AGTCCACCCA  
 21961 AAGCGTACAG GGGCCCAACT CGGCGCGCTG CGCATATTCT TGCTCGATGT TCTCCAGCG  
 22021 CTTTGGCAAC TGGCCCCAAA CTCCCATGGA TCACAACCCC ACCATGAACC TTATTACGGG  
 22081 GGTACCCAAC TCCATGCTCA ACAGTCCCAA GGTACAGCCC ACCCTGCGTC CGAACCAGGA  
 22141 ACAGCTCTAC AGCTTCTGCG AGCGCCACTC GCCTACTTTC CGCAGCCACA GTGCGCAGAT  
 22201 TAGGAGCGCC ACTCTTTTTT GTCACTTGA AAACATGTAA AATATATGTA CTAGAGACAC  
 22261 TTTCAATAAA GGCAATGTCT TTTATTTGTA CACTCTCGGG TGATTAATTA CCCCCACCT  
 22321 TGCCCTCTGC GCGCTTTAAA AATCAAGGG GTTCTGCGC GCATGCTAT TCTCCAGCTG  
 22381 CAGGAGACAG TTGCGATACT GGTGTTTAGT GCTCCACTTA AACTCAGGCA CAACCATCCG  
 22441 CGCGCACTCG GTGAAGTTTT CACTCCACAG GCTGCGCACC ATCAACCAAG CGTTTAGCAG  
 22501 GTCGGGCGCC GATATCTTGA AGTGCAGTGT GGGGCTCGG CCGTGTGCGC GCGAGTTGCG  
 22561 ATACACAGGG TTGCAGCACT GGAACACTAT CAGCGCGGG GTGTTGACG CTGCTAGCAG  
 22621 GCTCTTGTGC GAGATCAGAT CCGCGTCCAG GTCCCTCGCC TTGCTCAGG CGAACGGAT  
 22681 CACTTTGGT AGCTGCTTTC CCAAAAAGGG CGCTGTGCGC GGCTTTGAGT TGCACTCGCA  
 22741 CCGTAGTGGC ATCAAAAAGG GACCGTGCCC GGTCTGGGGG TTAGATATCA GCGCTGTGAT  
 22801 AAAAGCCTTG ATCTGCTTAA AAGCCACCTG AGCCTTTGCG CTTTACAGA AGAATATGCC  
 22861 GCAAGACTTG CCGGAAACTG GATTGGCCGG ACAGGCGCG TCGTGCAGCG AGCACCTTGC  
 22921 GTCGGTGTTG GAGATCTGCA CCACATTTGG GCCCCACCGG TTCTCACGA TCTTGGCCTT

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FIGURE 23  
(SHEET 7)

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22981 GCTAGACTGC TCCTTCAGCG CGCGCTGCC GTTTCGCTC GTCACATCCA TTTCATCAG  
 23041 GTGCTCCTTA TTATCATAA TGCTTCGGTG TAGACACTTA AGCTCGCCTT CGATCTCAGC  
 23101 GCAGCGGTGC AGCCACAACG CGCAGCCCGT GGGCTCGTGA TGCTTGTAGG TCACCTCTGC  
 23161 AAACGACTGC AGGTACGCCT GCAGGAATCG CCCCATCATC GTCACAAAGG TCTTTGTGCT  
 23221 GGTGAAGGTC AGCTGCAACC CGCGGTGCTC CTGCTTCAGC CAGGTCTTGC ATACGGCCGC  
 23281 CAGAGCTTCC ACTTGGTCAG GCAGTAGTTT GAAGTTCGCC TTTAGATCGT TATCAACGTC  
 23341 GTACTTGTCC ATCAGCGCGC GCGCAGCCTC CATGCCCTTC TCCCAACGAG ACACATCGG  
 23401 CACACTCAGC GGGTTCATCA CCGTAATTTT ACTTTCGCTT TCGCTGGGCT CTCTCTCTTC  
 23461 CTCTTGCCTC GCATATCCAC GCGCCACTGG GTGCTTCTCA TTCACGCCGC GCATCTGTGC  
 23521 CTTACTCTCT TTGCCATGCT TGATTAGCAC CGGTGGGTGT GTGAAGCCCA CCAATTGTAG  
 23581 CGCACATCTT TCTCTTTCTT CCTCGCTGTC CACGATTACC TCTGTGTAGT CGGGCGCTCA  
 23641 GGGCTTGGGA GAAGGGCGCT TCTTTTCTTT CTCTGGCGCA ATGGCCAAAT CCGCGCCGCA  
 23701 GGTGATGGCC CGCGGCTGG GTGTGCGCGT CACCAGCGCG TCTGTGTAGT AGTCTTCTCT  
 23761 GTCTCTCGAC TCGATACGCC GCTCATCTCG CTTTTTTGGG GCGCGCCGCG GAGGCGCGCG  
 23821 CGACGGGAGC GGGGACGACA GTCTCTCCAT GGTGGGGGGA CGTCGCGCGC CACCGCTTAC  
 23881 GCGCTCGGGG GTGGTTTCGC GCTGCTCTCT TTCCGACTGT GCCATTCTCT TCTCTATAG  
 23941 GCAGAAAAAG ATCATGGAGT CAGTCGAGAA GAAGGACAGC CTAACCGCCC CCTCTAGATT  
 24001 CGCACACACC GCTTCCACCG ATGCCGCCAA CGCGCTTACC ACCTTCCCGC TCGAGGCCAC  
 24061 CGCGCTTCAG GAGGAGGAAG TGATTATCGA GCAGGACCCA GGTTTTGTAA GCGAGAGACA  
 24121 CGAGGACCGC TCAGTACCAA CAGAGGATAA AAAGCAAGAC CAGGACACAG CAGAGGCAAA  
 24181 CGAGGAACAA GTCCGGCGGG GGGACGAAG GCATGCGCAC TACCTAGATT TGGGAGACGA  
 24241 CGTGCTGTGT AAGCATCTGC AGCGCCAGTG CGCCATTATC TGCGACGGCT TGCAGAGAGC  
 24301 CAGCGATGTG CCCCTCGCCA TAGCGGATGT CAGGCTTGGC TAGCAAGCGC ACCTATTCTC  
 24361 ACCGCGGTGA CCCCCAAAC GCGCAATGAG GCGCAGTGT GAGCCCAACC CCGCGCTCAA  
 24421 CTCTACCCCC GTATTGTCGG TGCCAGAGGT GCTTGGCCAC TATCATCTCT TTTTCCAAAT  
 24481 CTCGAAGATA CCCCTATCTC GCGGTGCCAA CGCGAGCCGA GCGGACAAGC AGCTGGCTTT  
 24541 GCGCGGAGCG GCTGTCTATC CTGATATCGC CTGCTCAAC GAAGTGCCTA AAATCTTTGA  
 24601 GGGCTTTGGA CGCGACGAGA AGCGCGCGCG AAACGCTCTG CAACAGGAAA ACAGCGAAAA  
 24661 TGAAGTTCAC TCTGGAATGT TGTGTGAATC CGAGGGTGAC AAACGCGCGC TAGCCGTATC  
 24721 AAAACGACAG ATCGAGGTCA CCCACTTTGC CTACCCGGCA CTTAACTCAT CCCCAGAGGT  
 24781 CATGAGCACA GTCATGAGTG AGCTGATCGT GCGCCGTGCG CAGCCCCCTG AGAGGATATC  
 24841 AAATTTCGCA GAACAAACAG AGGAGGGGCT ACCCGCAATT GCGCAGAGC CGCAACTCAA TGATGGCCGC  
 24901 TGCGCTTCAA ACGCGGAGC CTGCGGACTT GGTAGTGCAT GCAGCGGTCT TTTGCTGACC CCGAGATGCA  
 25021 GCGCAAGCTA GAGGAAACAT TGCCTATCAC TCTGCAACCT GGTCTCTCAT CTGTGAAATT TGCAGAAAA  
 25081 CAAGATCTCC AAGCTGGAGC TCTGCAACCT GGTCTCTCAT CTGTGAAATT TGCAGAAAA  
 25141 CGCGCTTGGG CAAAACGTGC TTCAITCCAC GCTCAAGGGC GAGGCGCGCC CGCTTTGGGG  
 25201 CGCGAGCTGC GTTTACTTAT TTCTATGCTA CACCTGGGAG ACGGCCATGG CGGTTTGGCA  
 25261 GCAGTGTCTG GAGGAGTGCA ACCTCAAGGA GCTGCAAGAA CTGCTAAAGC AAACTTTGAA  
 25321 GGAACCTATG ACGGCTTCA ACAGAGCGCT CGTGGCGCGC CACCTGGCGG ACATCATTTT  
 25381 CCGCAAGCGC CTGCTTAAAA CCGTCAACCA GGGTCTGCCA GACTTACACA GTCAAGGAT  
 25441 GTTGCAAGAC TTTAGGAAC TTATCTTAGA CGGCTCAGGA ATCTTGGCGC CCACCTGCTG  
 25501 TGCACTTCTT AGCGACTTTG TGCCCAATTA GTACCGGAA TGCCCTCGCG CGCTTTGGGG  
 25561 CCACTGTCTAC CTCTGCGAGC TAGCCAACTA CTTTGGCTAC CACTCTGACA TAATGAGAGA  
 25621 CGTAGAGCGT GACGGTCTAC TGGAGTGTCA CTGTGCTGAC AACCTTGTGC CCGCGCACGC  
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 26281 CGCGCGCGCC CCAAGAAATCG GCAACCGGTT CCAGCATGCG TACACCTCCG GCTCTCTCAG  
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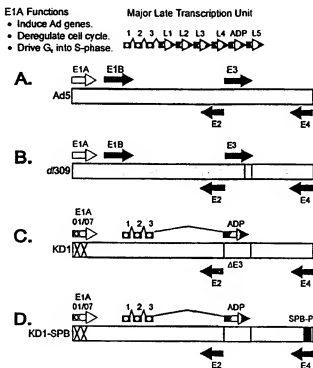


FIGURE 24

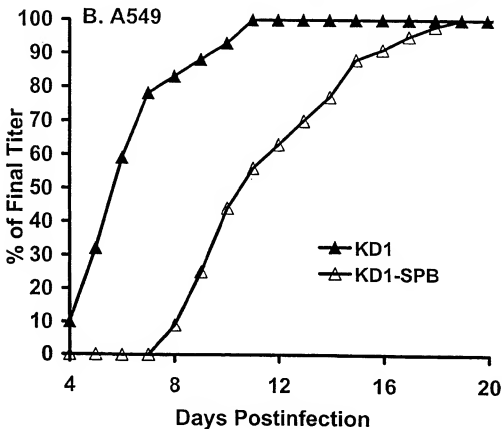
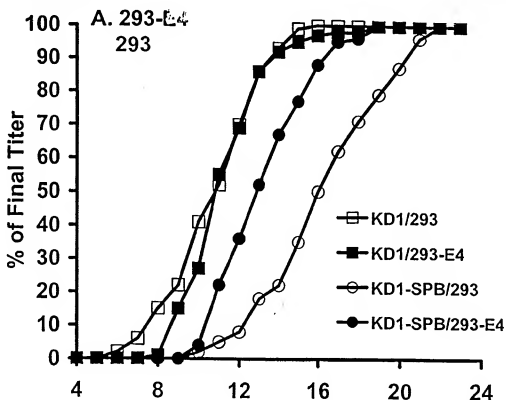


FIGURE 25

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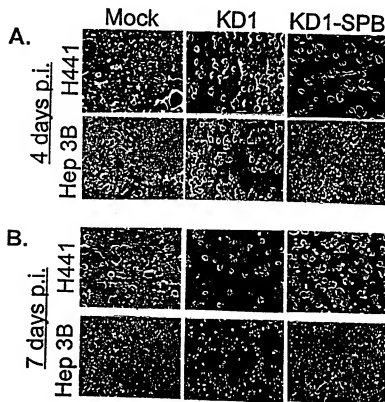
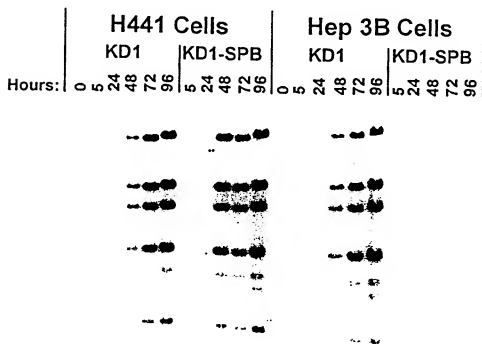


FIGURE 26



**FIGURE 27A**

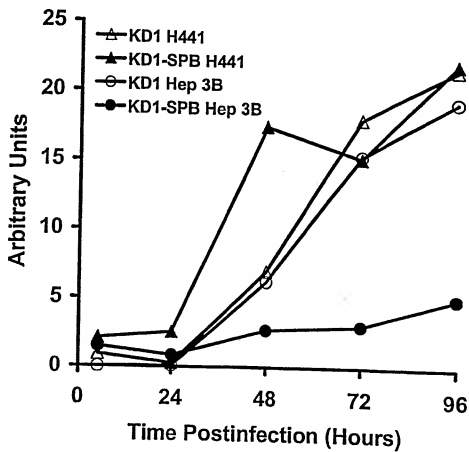


FIGURE 27B

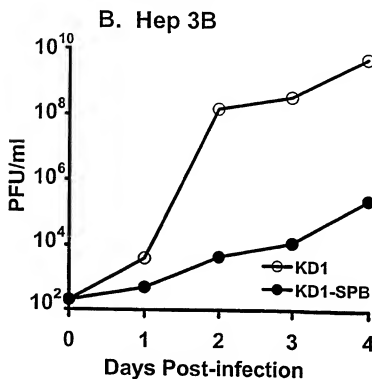
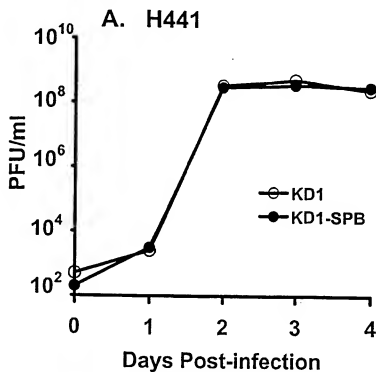


FIGURE 28

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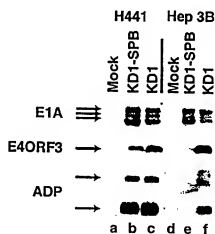


FIGURE 29

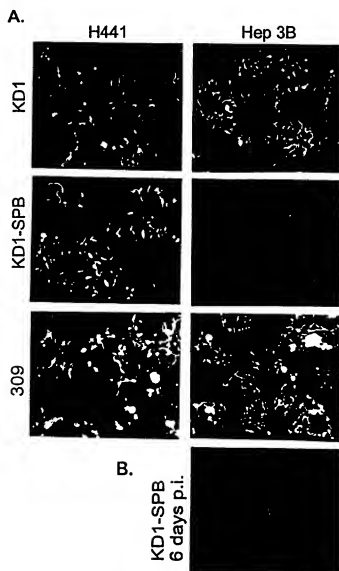


FIGURE 30

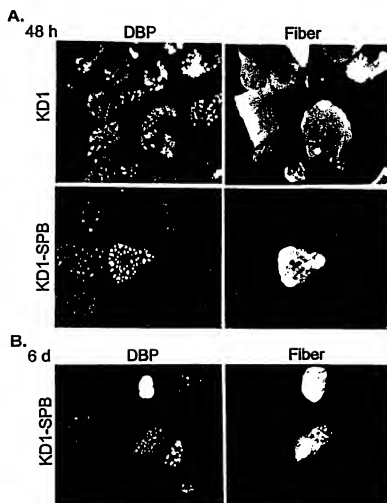


FIGURE 31

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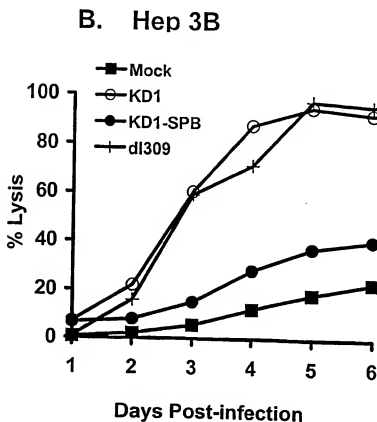
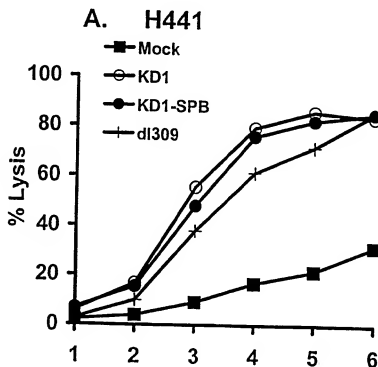


FIGURE 32

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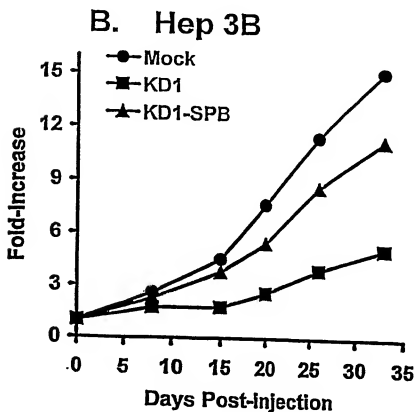
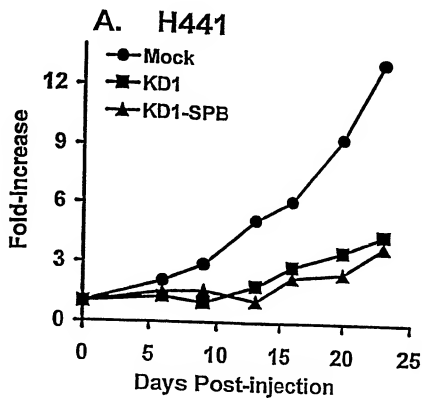


FIGURE 33

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&lt;160&gt; 20

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&lt;212&gt; DNA

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&lt;400&gt; 1

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Leu Gly Met Trp Trp Phe Ser Ile Ala Leu Met Phe Val Cys Leu Ile
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Ile Tyr Lys Pro Ile Ile Val Leu Asn Pro Asn Asn Asp Gly Ile His
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Val Asn Asp Trp Ala Ser Leu Asp Met Trp Trp Phe Ser Ile Ala Leu
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Met Phe Val Cys Leu Ile Ile Met Trp Leu Ile Cys Cys Leu Lys Arg
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Arg Arg Ala Arg Pro Pro Ile Tyr Arg Pro Ile Ile Val Leu Asn Pro
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Met Trp Leu Ile Cys Cys Leu Lys Arg Lys Arg Ala Arg Pro Pro Ile  
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&lt;213&gt; Adenovirus subgroup C

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Leu Gly Met Trp Trp Phe Ser Ile Ala Leu Met Phe Val Cys Leu Ile  
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&lt;213&gt; Adenovirus subgroup C

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Val Asn Asp Trp Ala Ser Leu Asp Met Trp Trp Phe Ser Ile Ala Leu  
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